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THE DETERMINATION OF SODIUM IN BIOLOGICAL FLUIDS

by

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submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

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FALCON BOND

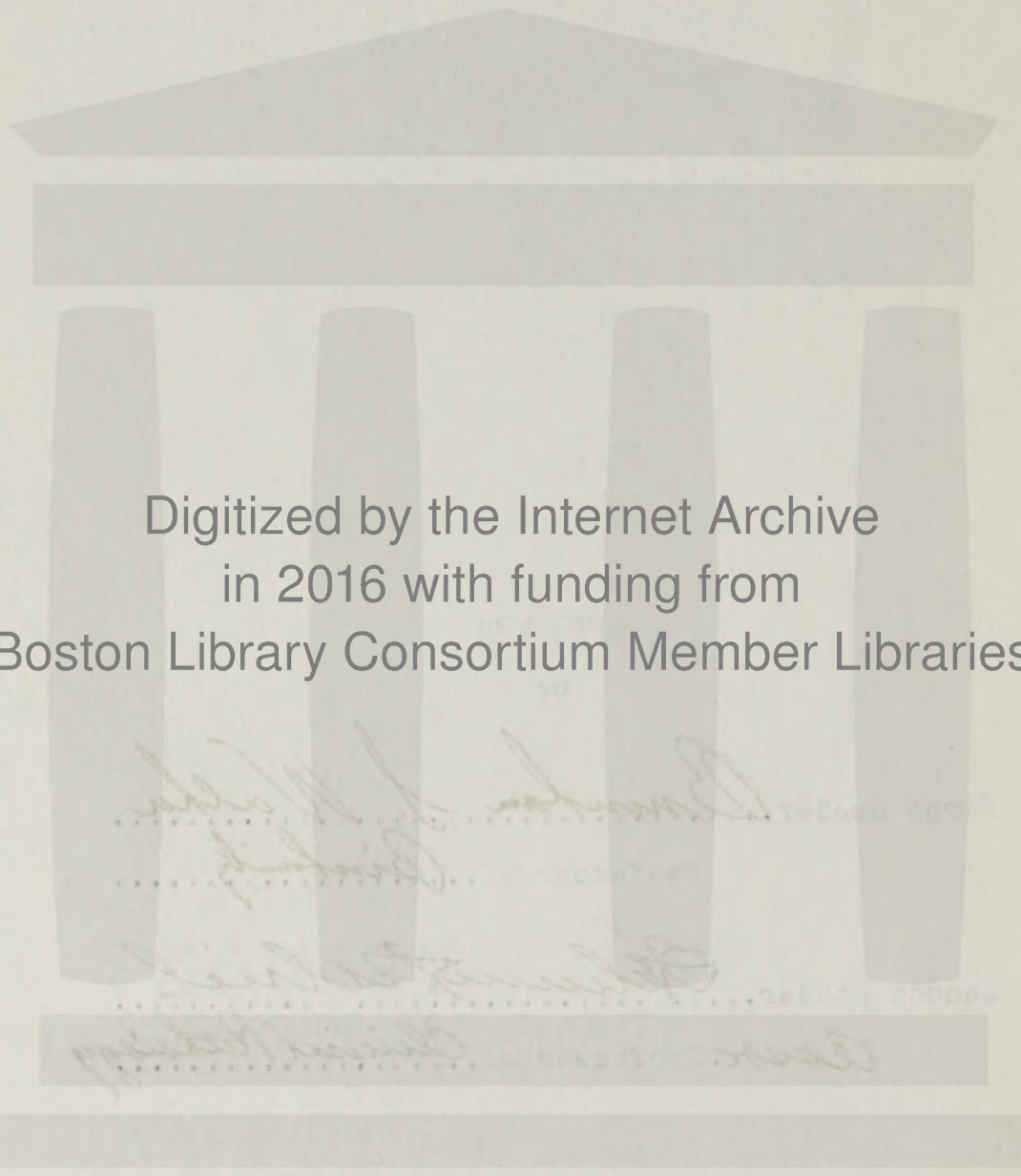
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4. Conformity with Beer's law of color with
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5. Variation of $\frac{\text{density}}{\text{concentration}}$ with density.
First solution

6. Variation of $\frac{\text{density}}{\text{concentration}}$ with density.
Second solution

7. Conformity with Beer's law over short range of
concentration

Photograph

1. The reactor

Introduction

Recent work on sodium metabolism in adrenal insufficiency, disturbed acid-base equilibrium, and forced loss of fluids (57,58) has changed the status of the determination of sodium in biological fluids from a test rarely done in the clinical laboratory to one that is rapidly becoming necessary in the armamentarium of the average clinical laboratory technician. Although cases have been reported in which twenty-five percent or more of the sodium of the body has been lost (59), the body generally maintains homeostasis with respect to sodium to such an extent that deviations in the sodium content of body fluids of four percent from the mean normal figure may be presumed to be indicative of a beginning pathological process (36). Thus, to be of any value, a method of determination of sodium must be accurate to one percent. This contribution represents an attempt to develop a method, with a maximum error of one percent, which shall be simple enough to be performed in routine clinical laboratories.

Review of Literature

Methods not employing uranyl triple salts

The classical method for the determination of sodium requires the removal of all ions but sodium and potassium, the precipitation of sodium and potassium as chlorides, the separation of potassium as chloroplatinate or perchlorate, and the calculation of sodium by difference (⁷²₉₄). McLean and Van Slyke (62), in 1915, devised a method for titration of chloride by adding an excess of silver nitrate and titrating the excess with potassium iodide. McCrudden and Sargent (61) applied this titration to the determination of sodium; by precipitating both sodium and potassium as chlorides and determining the chloride content of the weighed precipitate, they calculated the percentage of sodium in the precipitate. These authors pointed out that any error in technic is magnified in calculation.

Fenton (28), in 1898, proposed a method for the determination of sodium, based on the insolubility of the sodium salt of dihydroxytartaric acid in cold water. This method has not been used to any great extent.

Ball (5) introduced a gravimetric method precipitating sodium as the complex bismuth caesium nitrite having the composition $9\text{CsNO}_2 \cdot 6\text{NaNO}_2 \cdot 5\text{Bi}(\text{NO}_2)_3$. This method was modified and improved by Doisy and Bell (22), who determined the nitrite content of the precipitate volumetrically and colorimetrically.

Kramer (47) precipitated sodium as the pyroantimonate $\text{Na}_2\text{H}_2\text{Sb}_2\text{O}_7 \cdot 6\text{H}_2\text{O}$ in alcoholic medium, and weighed the precipitate. This gravimetric method was used by Kramer and Tisdall in several studies (49, 50, 92,) of sodium in biological fluids. In 1924 Bálint (3) and, independently, Kramer and Gittleman (48) adapted the pyroantimonate precipitation to a volumetric procedure by adding potassium iodide to the dissolved precipitate and titrating the liberated iodine. The method was refined by Kerr (42) and by Rourke (79), who removed protein from the serum or plasma before adding the pyroantimonate reagent. This iodometric method has been applied to milk by Sato and Murata (84). Lewin (53) dissolved the precipitated pyroantimonate in HCl , reduced the antimony with sodium sulfite, boiled off the excess^{of}/sulfur dioxide, and titrated with standard bromate solution. A colorimetric method, based on the measurement of the orange color of antimony trisulfide obtained from the pyroantimonate precipitate, was devised by Yoshimatsu (98).

Stoddard (89) precipitated out the alkaline earths, electrolyzed the remaining solution in two connected tubes of mercury, dissolved the sodium and potassium out of the resulting cathode amalgam, and titrated with standard acid. The value of potassium, determined by the method of Fiske and Litarczek (29), when subtracted from the value of sodium plus potassium obtained from the titration, gave the figure for sodium.

Brown and Shohl (10) determined total base in blood by the method of Stadie and Ross (28); then by quantitation of other cations, they obtained a figure for sodium plus potassium.

Thomson and Lee (91) adapted spectrographic analysis, as developed by Russanov(80), Jansen, Heyes, and Richter (38), and Buffendack, Wolfe, and Smith(23), to biological fluids.

Prinsen-Geerlings (76) in 1937 published an article on the determination of serum sodium by the polarograph, an instrument which registers current voltage lines automatically; no figures were reported in the paper, so no evaluation of the method is possible.

Methods employing uranyl triple salts

Since 1886, when Streng (90) isolated and identified a triple acetate of uranium, magnesium, and sodium, a series of similar uranyl-sodium-divalent metal acetates has been prepared. They possess the common characteristics of easy solubility in water and sparing solubility in alcohol. Kolthoff (45) prepared the zinc salt $(\text{UO}_2)_3\text{ZnNa}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$ and used its formation as a qualitative test for sodium. Caley (13) prepared the corresponding cobalt salt and used cobalt uranyl acetate as a qualitative test for sodium. The eleventh United States Pharmacopoeia (73) also has adopted this test. Feldstein and Ward (27) used nickel uranyl acetate as a qualitative reagent for sodium. Chang

and Tseng (18) have used the manganous salt in quantitative determinations. There is concurrence among the users of these triple salts that the cobalt, manganese, zinc, and nickel salts are isomorphic, having the formula $(\text{UO}_2)_3 \text{Na-M}^{++} (\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$, where M^{++} represents the divalent metal. There is strong disagreement over the composition of the magnesium salt. Streng (90) and Blanchetière (9) stated its composition as $(\text{UO}_2)_3 \text{MgNa}(\text{CH}_3\text{COO})_9 \cdot 9\text{H}_2\text{O}$; Caley and Foulk (17) assigned 6.5 molecules of water of crystallization to it; Nydahl (66), Kahane (40), and Raszeja (77, 78) 8 molecules of water; and Miholic (63), Barber and Kolthoff (6), and Alten, Wieland, and Hille (2), 6 molecules.

After one of the triple salts has been separated from the solution, the quantitation may proceed gravimetrically, volumetrically, or colorimetrically.

All gravimetric methods involve the washing, drying, and weighing of the isolated triple salt. The precipitate is washed first with alcohol (6) or glacial acetic acid (82) saturated with the triple salt, then with ether. The manganese and zinc salts may be dried in air, without the use of desiccators or ovens. The uncertainty of composition of the magnesium salt makes it necessary for each person using it to adhere to his own set of standard conditions for maintaining constant composition of the precipitates.

The gravimetric method using the magnesium triple salt, as developed by Streng (90), Miholic (63), Blanchetière (9),

Caley and Foulk (¹⁴₁₇), Kahane (40), and Kolger (44), has been found inaccurate by Kling and Lassieur (43) and by Barber and Kolthoff (6). Caley (16) has improved the method and limited the errors to about ± 1.5 percent.

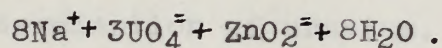
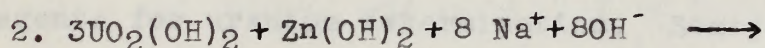
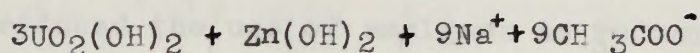
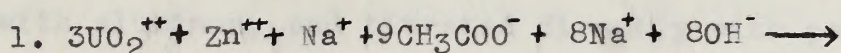
Barber and Kolthoff (6) developed a gravimetric method based on the weighing of the zinc triple salt, and demonstrated the stability of composition and ease of washing and drying of the precipitate. Later (7) they showed that the chief interfering substances are potassium, phosphate, and arsenate. Butler and Tuthill (12) applied the method to biological fluids and obtained excellent results after destruction of proteins. Jendrassik and Dziobek (39) adapted the method to the analysis of minute quantities of biological fluids by use of a sensitive torsion balance.

Chang and Tseng (18) found that uranyl manganese acetate is as satisfactory a reagent for the gravimetric determination of sodium as is uranyl zinc acetate.

The first volumetric determination of sodium based on triple salt separation was devised in 1930 by Caley (15), who dissolved the precipitate of uranyl magnesium sodium acetate and titrated the uranium with a standard phosphate solution till paper impregnated with potassium ferrocyanide showed no red color when dipped in the titration mixture. Rusznyák and Hatz (81) devised a similar procedure for the zinc triple salt. They added an excess of phosphate to precipitate the zinc and the uranium and titrated the excess phosphate with standard uranyl acetate solution.

Ufer and Kowik (12), Kahan (40), and Kojer (41), has
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 nation of sodium as its uranyl zinc acetate.
 The first volumetric determination of sodium based on
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 dissolved the precipitate of uranyl phosphate sodium acetate
 and filtered the supernatant and reprecipitated sodium
 with paper impregnated with potassium ferrioxalate, showed no
 red color when placed in the titration mixture. Hargrave
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 triple salt. They added an excess of phosphate to precipi-
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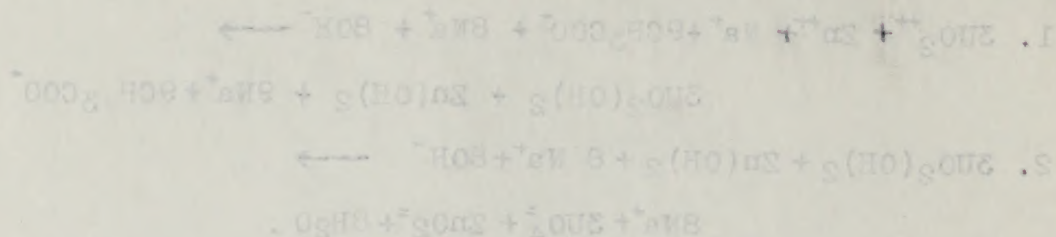
In the volumetric method of Dobbins and Byrd (21) an excess of sodium hydroxide is added, converting the uranium and zinc in the zinc triple salt to the amphoteric hydroxides, according to the equations



By titrating the excess sodium hydroxide with standard acid to a phenolphthalein end point, reaction 2 is entirely inhibited and a stoichiometric relationship exists between the amount of sodium hydroxide consumed and the amount of triple salt present. Sassier (83) used this method for the determination of sodium in urine. Weinbach (96), in applying the method to biological fluids, titrated the zinc and uranium directly with sodium hydroxide to a phenolphthalein end point.

It has long been known that uranyl compounds can be reduced from the hexavalent to the tri- and tetravalent states in acid solution, and that any trivalent uranium can be oxidized to the tetravalent state by aeration (52). This reaction was applied to the zinc triple salt by Gall and Heinig (31), who reduced with electrolytic cadmium, aerated, and titrated the tetravalent uranium with permanganate. Chen (19) used a similar procedure. Furman, Caley, and Schoonover (30) precipitated sodium as the magnesium triple salt, reduced the uranium by passing the acid solution

In the volumetric method of Dobbins and Byrd (21) an excess of sodium hydroxide is added, converting the uranium and zinc in the zinc triple salt to the amphoteric hydroxides according to the equations



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It has long been known that uranyl compounds can be reduced from the hexavalent to the tri- and tetravalent states in acid solution, and that any trivalent uranium can be oxidized to the tetravalent state by ceric (24). This reaction was applied to the zinc triple salt by Bell and Reine (25), who reduced with electrolytic sodium, oxidized, and titrated the tetravalent uranium with potassium dichromate. Chen (26) used a similar procedure. Burton, Galey, and Schoonover (27) precipitated uranyl as the magnesium triple salt, reduced the uranium by passing the acid solution

through a Jones reductor, aerated, added an excess of ceric sulfate, and back-titrated with ferrous sulfate. Kahane (40) precipitated the magnesium salt, dissolved in acid, reduced with aluminum, and titrated directly with permanganate.

Kano (41) introduced the use of amalgams as reducing agents for uranium determination. Someya (87) used bismuth amalgam for the determination of uranium. He worked out a method for sodium determination in which he precipitated with uranyl magnesium acetate, dissolved the precipitate in acid, reduced with bismuth amalgam, aerated, and titrated with potassium dichromate. Kolthoff and Lingane (46) used a similar method, employing the zinc triple salt, zinc amalgam, and dichromate. Raszeja (78) precipitated the sodium of blood as the magnesium triple salt, reduced with zinc amalgam, and titrated with permanganate. Ball and Sadusk (4) used the Kolthoff and Lingane method for determining blood sodium, and Holmes and Kirk (37) used cadmium amalgam as the reductant and ceric sulfate as the oxidant.

Volumetric procedures have also been worked out for the determination of zinc in zinc uranyl sodium acetate (51) and for acetate in magnesium uranyl sodium acetate (24).

The first reliable colorimetric test for the detection of uranyl compounds was published by Bruttini (11) in 1893. It depends on the formation of a reddish brown uranyl potassium ferrocyanide when potassium ferrocyanide is added to an acid solution of a uranyl salt. In 1927, after the application

through a Jones reductor, heated, added an excess of ceric
sulfate, and back-titrated with ferrous sulfate. Kahan (40)
precipitated the manganous salt, dissolved in acid, reduced
with aluminum, and titrated directly with permanganate.
Kane (41) introduced the use of amalgams as reducing
agents for uranium determination. Somaya (37) used bismuth
amalgam for the determination of uranium. He worked out a
method for sodium determination in which he precipitated with
ammonium manganous acetate, dissolved the precipitate in acid,
reduced with bismuth amalgam, heated, and titrated with
potassium dichromate. Kolichoff and Lingane (42) used a similar
method, employing the zinc-triglycinate salt, zinc amalgam, and
dichromate. Krasaja (38) precipitated the sodium of blood
as the manganous triglycinate salt, reduced with zinc amalgam, and
titrated with permanganate. Ball and Sabus (43) used the
Kolichoff and Lingane method for determining blood sodium, and
Holmes and Kirk (37) used carbon amalgam as the reductant
and ceric sulfate as the oxidant.
Volometric procedures have also been worked out for the
determination of zinc in zinc amyl sodium acetate (31) and
for acetate in magnesium amyl sodium acetate (34).
The first reliable colorimetric test for the detection
of uranyl compounds was published by Baerlein (11) in 1908.
It depends on the formation of a reddish brown uranyl potas-
sium ferrocyanide when potassium ferrocyanide is added to an
acid solution of a uranyl salt. In 1907, after the application

of the uranyl magnesium acetate reagent to the determination of sodium by Blanchetière (9), Barrensheen and Messiner (8) developed a colorimetric method for the determination of blood sodium based on the formation of the ferrocyanide of the uranium in the magnesium triple salt. Poulsson (74) and Tissier and Bénard (93) used similar methods. McCance and Shipp (60) used the ferrocyanide color from the zinc triple salt as a measure of blood sodium. Their method was adapted to other biological fluids by Salit (82), Alten and Wieland (1), Marenzi and Gerschmann (55, 56) Malyarov and Yudenich (54), and Oberst (67).

Caley and Foulk (17), at the outset of their work with uranyl magnesium sodium acetate, appreciated the possibility of measuring the intensity of the yellow color of solutions of the salt as a quantitative procedure. Hoffman and Osgood (36), using uranyl zinc sodium acetate, measured the light absorption in a photoelectric colorimeter (85) after stabilizing the color with ammonium thiocyanate.

Müller (65), in 1919, discovered that sodium salts of alpha-hydroxy and alpha-keto acids and of aromatic hydroxy carboxylic acids give yellow or red colors with uranyl salts in the absence of free mineral acid. Elías (25) developed a colorimetric micromethod for determining sodium by dissolving the precipitate of magnesium uranyl sodium acetate and developing the color with sodium salicylate.

Das Gupta (20) found that phenolic acids generally give

of the urinary magnesium acetate present to the determination of sodium by Harnad (3), Harnad and Messner (4) developed a colorimetric method for the determination of blood sodium based on the formation of the ferrocyanide of the uranium in the magnesium triple salt. Poulsson (5) and Tjander and Bernald (6) used similar methods. McGowan and Ship (7) used the ferrocyanide color from the same triple salt as a measure of blood sodium. Their method was adapted to other biological fluids by Miller (8), Allen and Wilson (9), Wenzel and Gerschwann (10, 11), Wajsborn and Yatschik (12), and Bernald (13).

Calley and Smith (14), at the outset of their work with urinary magnesium sodium acetate, appreciated the possibility of measuring the intensity of the yellow color of solutions of the salt as a quantitative procedure. Hollman and Haged (15), using urinary magnesium sodium acetate, measured the light absorption in a photoelectric colorimeter (16) after stabilizing the color with ammonium thiocyanate.

Miller (17), in 1919, discovered that both in salts of alpha-hydroxy and alpha-keto acids and of aromatic hydroxy carboxylic acids give yellow or red colors with urinary salts in the absence of free mineral acids. Miller (18) developed a colorimetric method for determining sodium by dissolving the precipitate of magnesium urinary sodium acetate and developing the color with sodium sulfide.

Das Gupta (19) found that phenolic acids generally give

colors with uranyl salts and that sodium acetate intensifies the colors. He recommended gallic and tannic acids as colorimetric reagents for uranium.

Ogden (68) used cochineal as an external indicator for excessive uranium in the titration of phosphate with uranyl acetate. Germuth and Mitchell (32) propose sodium alizarin sulfonate as a color reagent for uranium.

Woelfel (97) has recently devised a colorimetric method for the determination of sodium, based on precipitation with uranyl manganese acetate, oxidation of the manganese to permanganate, and measuring the color.

colors with weakly acidic and weakly basic substances
the colors. As recommended for the colors as color-
metric reactions for drinking.

Agden (53) used cobalt as an external indicator for
excessive reaction to the detection of phosphates with arsenic
acetate. Garman and Mitchell (54) proposed cobalt arsenic
acetate as a color reaction for drinking.

Mitchell (55) has recently devised a colorimetric method
for the determination of arsenic, based on precipitation with
arsenic molybdate acetate, oxidation of the molybdenum to per-
manganate, and measuring the color.

Experimental

Choice of Method

Since the introduction of the photoelectric colorimeter into biochemical laboratories (26,85), the speed and ease of operation of the instrument and the minimum of subjective errors entailed in its use have made the photoelectric measurement of color very popular. The simplification of any method of analysis without the sacrifice of accuracy is the goal of all analysts. By a simple survey of the limits of accuracy of the Evelyn (26) photoelectric colorimeter, it can be shown that the error involved in the use of the instrument can be held to .75%.

Getman and Daniels (33) state Beer's law as follows:

$$I = I_0 e^{-k'lc}$$

where

I_0 is the intensity of light striking the absorbing medium,

I is the intensity of light after having passed through a depth l of the medium,

c is the concentration of the absorbing medium,

e is the base of natural logarithms, and

k' is a constant characteristic of the absorbing material and of the wave length of the light passing through.

In the Evelyn colorimeter the measurements of absorption are always made through standard tubes, so that l is likewise constant. The tube containing the colored material is placed between a lamp and a photocell, so that the transmitted light

Experimental

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Beeman and Daniels (23) state Beer's law as follows:

$$I = I_0 e^{-K'cl}$$

where I_0 is the intensity of light striking the absorbing medium,

I is the intensity of light after having passed through a depth l of the medium,

c is the concentration of the absorbing medium,

e is the base of natural logarithms, and

K' is a constant characteristic of the absorbing

material and of the wave length of the light passing through.

In the Evelyn colorimeter the measurements of absorption are always made through standard tubes, so that l is likewise constant. The tube containing the colored material is placed between a lamp and a phototube, so that the transmitted light

falls on the photocell. The cell is connected to a galvanometer, the scale of which is graduated into one hundred equal parts; the maximum error of reading is one quarter of a scale division. Before making the determination, the galvanometer is adjusted to read 100 when a tube containing pure solvent (reaction mixture minus the substance to be determined; that is, zero concentration of the colored substance) is placed between the lamp and the photocell. Then, when the tube containing the colored substance is placed between the lamp and the cell, the light transmitted, as measured in percentage of that transmitted by the blank tube, is given directly by the galvanometer reading. The transmission of the sample solution, defined as the ratio of the light transmitted by the sample to that transmitted by the blank, is given by the expression

$$T = \frac{G}{100},$$

in which T is the transmission and G is the galvanometer reading of the sample solution.

Since the concentration of colored substance in the blank tube is zero, then by Beer's law the intensity of light transmitted by the blank tube

$$I_b = I_0 e^{-k'l_0}$$

or $I_b = I_0 e^0.$

Since $e^0 = 1,$

$$I_b = I_0.$$

Now considering the intensity of light transmitted by the sample, $I_x = I_0 e^{-k'l_c}.$

cell on the photocell. The cell is connected to a galvanometer, the scale of which is graduated into one hundred equal parts; the maximum error of reading is one quarter of a scale division. Before making the determination, the galvanometer is adjusted to read 100 when a tube containing pure solvent (reaction mixture minus the substance to be determined; that is, zero concentration of the colored substance) is placed between the lamp and the photocell. Then when the tube containing the colored substance is placed between the lamp and the cell, the light transmitted, as measured in percentage of that transmitted by the blank tube, is given directly by the galvanometer reading. The transmission of the sample solution, defined as the ratio of the light transmitted by the sample to that transmitted by the blank, is given by the expression

$$T = \frac{I}{I_0} \times 100$$

in which T is the transmission and I is the galvanometer reading of the sample solution.

Since the concentration of colored substance in the blank tube is zero, then by Beer's law the intensity of

light transmitted by the blank tube

$$I_0 = I_0 e^{-k' \cdot 0}$$

$$I_0 = I_0 e^0$$

$$e^0 = 1$$

$$I_0 = I_0$$

Now considering the intensity of light transmitted by the sample, $I = I_0 e^{-k' \cdot c}$

The ratio of light transmitted by sample and blank

$$T = \frac{I_x}{I_b} = \frac{I_0 e^{-k'lc}}{I_0} = e^{-k'lc}$$

And since $T = \frac{G}{100}$,

$$\frac{G}{100} = e^{-k'lc}$$

Passing to common logarithms,

$$\log G - \log 100 = -k' l c \log e.$$

Since k' , l , and $\log e$ are constants, they can be combined to form a new constant K .

$$\text{Then } \log G - \log 100 = -Kc$$

$$\text{or } \log 100 - \log G = Kc$$

$$\text{or } c = \frac{2 - \log G}{K}$$

L , the density defined as $(2 - \log G)$, is tabulated against G for different readings of G , and c is calculated from the formula,

$$\frac{L}{K} = c.$$

K is determined for any colored substance which follows Beer's law by calculating values of L for a series of observed G values and plotting the values of L against the corresponding known values of c .

The calculations in Table 1 show the percentage^{of} error due to inaccuracy in reading of one quarter scale division at different points on the scale when aliquot samples are varied so that the reading corresponds in every case to a constant concentration of chromogen in the original material.

The ratio of light transmitted by sample and blank

$$T = \frac{I_x}{I_0} = \frac{10^{-k'lc}}{10^{-k''lc}} = e^{-k'lc + k''lc}$$

$$\text{And since } T = \frac{G}{100}$$

$$\frac{G}{100} = e^{-k'lc + k''lc}$$

Passing to common logarithms,

$$\log G - \log 100 = -k'lc + k''lc$$

Since k' , l , and $\log e$ are constants, they can be combined

to form a new constant K .

$$\text{Then } \log G - \log 100 = -Kc$$

$$\text{or } \log 100 - \log G = Kc$$

$$\text{or } c = \frac{2 - \log G}{K}$$

I , the density defined as $(2 - \log G)$, is tabulated

against G for different readings of G , and c is calculated

from the formula,

$$\frac{I}{K} = c$$

K is determined for any colored substance which follows

Beer's law by calculating values of I for a series of op-

posed G values and plotting the values of I against the

corresponding known values of c .

The relations in Table I show the percentage error

due to inaccuracy in reading of one quarter scale division

at different points on the scale when aliquot samples are

varied so that the reading corresponds in every case to a

constant concentration of chromogen in the original material.

Table 1
Percentage error involved in inaccuracy of reading galvanometer

G (galvanometer reading)	$L_G =$ $2 - \log G$	$G + \frac{1}{4}$	$L_{G + \frac{1}{4}}$	$\Delta L =$ $L_G - L_{G + \frac{1}{4}}$	% Error = $\frac{\Delta L}{L_G} \times 100$
1.00	2.00000	1.25	1.91309	.08691	4.346
10.00	1.00000	10.25	.98928	.01072	1.072
12.00	.92082	12.25	.91186	.00896	.985
20.00	.69897	20.25	.69357	.00540	.744
25.00	.60206	25.25	.59774	.00432	.718
30.00	.52288	30.25	.51927	.00361	.691
35.00	.45593	35.25	.45284	.00309	.678
38.00	.42022	38.25	.41737	.00285	.678
40.00	.39794	40.25	.39523	.00271	.682
45.00	.34679	45.25	.34438	.00241	.694
50.00	.30103	50.25	.29886	.00217	.722
60.00	.22185	60.25	.22004	.00181	.816
70.00	.15490	70.25	.15335	.00155	1.001
80.00	.09691	80.25	.09555	.00136	1.405
90.00	.04576	90.25	.04455	.00121	2.644
99.00	.00436	99.25	.00327	.00109	24.96

Table 1
Percentage error involved in inaccuracy of reading galvanometer

Galvanometer reading	$I_0 = 5-10 \times 10^{-6}$	2×10^{-6}	10^{-6}	$\Delta I = I_0 - I_1$	$\frac{\Delta I}{I_0} \times 100$	% Error
1.00	5.00000	1.25	1.2500	.08891	1.778	1.778
10.00	1.00000	10.25	.2500	.01072	1.072	1.072
12.00	.92082	12.25	.2682	.00896	.968	.968
20.00	.68827	20.25	.2623	.00540	.774	.774
25.00	.60208	25.25	.2674	.00423	.718	.718
30.00	.52388	30.25	.2667	.00361	.681	.681
35.00	.45292	35.25	.2634	.00308	.678	.678
38.00	.42022	38.25	.2673	.00283	.678	.678
40.00	.39794	40.25	.2632	.00271	.668	.668
45.00	.34878	45.25	.2643	.00241	.684	.684
50.00	.30102	50.25	.2658	.00217	.732	.732
60.00	.23198	60.25	.2604	.00161	.676	.676
70.00	.16480	70.25	.2622	.00123	1.001	1.001
80.00	.09891	80.25	.2638	.00128	1.406	1.406
90.00	.04878	90.25	.2648	.00121	2.444	2.444
95.00	.00822	95.25	.2627	.00108	24.98	24.98

It can be seen that the error is at a minimum of about .7% when the galvanometer reads between 25 and 50, and that the error is less than 1% when the galvanometer reads between 12 and 60.

If G is the correct galvanometer reading for a concentration c of chromogen in the sample, and $L_G = 2 - \log G$, then $L_G/c = K$ for that chromogen. If an error of one quarter scale division is made in reading the galvanometer, then $G + \frac{1}{4}$ is the erratic galvanometer reading, $L_{G+\frac{1}{4}}$ is the corresponding density, and $\left(\frac{L_{G+\frac{1}{4}}}{K}\right) = c'$ the erratic value for the concentration of chromogen obtained. The percentage of error caused by an erroneous galvanometer reading amounting to one quarter scale division is $\% \text{ error} = \frac{c - c' \times 100}{c}$

$$\begin{aligned}
 &= \frac{\left\{ \left(\frac{L_G}{K} \right) - \left(\frac{L_{G+\frac{1}{4}}}{K} \right) \right\} \times 100}{\frac{L_G}{K}} \\
 &= \frac{\left\{ \left(\frac{L_G}{K} - \frac{L_{G+\frac{1}{4}}}{K} \right) \right\} \times 100}{\frac{L_G}{K}} \\
 &= \frac{\left(L_G - L_{G+\frac{1}{4}} \right) \times K \times 100}{L_G} \\
 &= \frac{\left(L_G - L_{G+\frac{1}{4}} \right)}{L_G} \times 100
 \end{aligned}$$

These calculations show that the errors of the colorimeter are independent of the value of K ; that is, regardless of the size of the proportionality constant between the logarithm of the galvanometer reading and the absolute value of the concentration of chromogen in the sample, the accuracy of the reading can be held constant by diluting or concentrating the

It can be seen that the error is at a minimum of about .7% when the galvanometer reads between 35 and 50, and that the error is least when the galvanometer reads between 45 and 50.

If C is the correct galvanometer reading for a concentration of chromogen in the sample, and C_1 is the reading of the galvanometer when the error is one quarter of C , then the error is $C - C_1$. The percentage of error caused by this error is $\frac{C - C_1}{C} \times 100$. The percentage of error caused by this error is $\frac{C - C_1}{C} \times 100$.

$$\frac{\left(\frac{1}{C} - \frac{1}{C_1} \right) \times 100}{\frac{1}{C} - \frac{1}{C_1} \times 100} = \frac{\left(\frac{1}{C} - \frac{1}{C_1} \right) \times 100}{\frac{1}{C} - \frac{1}{C_1} \times 100}$$

These calculations show that the error of the colorimeter is independent of the value of K ; that is, regardless of the size of the proportionality constant between the logarithm of the galvanometer reading and the absolute value of the concentration of chromogen in the sample, the accuracy of the reading can be held constant by dividing or multiplying the

sample, provided that the color follows Beer's law. Thus, if the color developed by a certain reagent from the sodium in 1.0 cc. of blood gives a galvanometer reading of 1, the percentage of error would be 4.35. By using 0.5 cc. of blood, or by using twice the volume to contain the color from 1.0 cc., the reading obtained would be 10, and the percentage of error only 1.07.

It also follows that from the point of view of the colorimetry, there is no choice between two colors which follow Beer's law. Practically, of course, the choice must be limited to a color which gives accurate readings (near the middle of the galvanometer scale) with a conveniently sized sample or a conveniently and accurately obtainable aliquot portion of it.

In order to obtain results of the accuracy indicated in table 1, it is necessary that the colorimetric tubes be rigorously standardized. The tubes used in these experiments were standardized with filter 440, first by selecting tubes which gave identical readings when filled with distilled water, then by further selecting those tubes which gave identical readings when filled with a standard solution of the colored substance to be used in further experiments.

The methods so far proposed for the determination of sodium may be classified into six groups as follows:

1. Methods requiring multiple determinations, such as the methods of McCrudden and Sargent (61), Stoddard (98),

results, provided that the color follows Beer's law. Thus, if the color developed by a certain amount from the solution in 1.0 cc. of blood gives a colorimeter reading of 1, the percentage of error would be 4.55. By using 0.5 cc. of blood, or by using twice the volume to obtain the color from 1.0 cc., the reading obtained would be 10, and the percentage of error only 1.07.

It also follows that from the point of view of the colorimetry, there is no choice between two colors which follow Beer's law. Practically, of course, the choice must be limited to a color which gives accurate readings from the middle of the colorimeter scale) with a conveniently sized sample or a colorimetrically and accurately obtainable aliquot portion of it. In order to obtain results of the accuracy indicated in Table I, it is necessary that the colorimetric tubes be rigorously standardized. The tubes used in these experiments were standardized with filter 440, filled by automatic tubes which gave identical results when filled with distilled water, then by further selection those tubes which gave identical readings when filled with a standard solution of a colored substance to be used in further experiments.

The methods so far proposed for the determination of

solids can be classified into two groups as follows:

1. Methods requiring suitable determinations, and

as the methods of Kjeldahl and Gerhardt (21), Stricker (22),

Brown and Shohl (10), and Prinsen-Geerlings (75).

2. The dihydroxytartaric acid method of Fenton (28).

3. Spectrographic methods, as those of Thomson and Lee (91) and Jansen, Heyes, and Richter (38).

4. Methods using bismuth caesium nitrite (5,22).

5. Pyroantimonate methods, as those of Kramer (47), Kramer and Gittleman (48), and Lewin (53).

6. Uranium methods, as those of Blanchetière (9), Barber and Kolthoff (6), and Woelfel (97).

For routine clinical use multiple gravimetric analyses are too tedious. Fenton's (28) method was gravimetric. Since the spectrograph is not a commonly used instrument in clinical laboratories, a spectrographic method would have limited application. Doisy and Bell (22) in their development of the bismuth caesium nitrite method were unsuccessful in their efforts to stabilize the reagents. As a volumetric procedure, Rourke's (79) modification of the Kramer and Gittleman (48) pyroantimonate method seems above reproach. But the instability of arsenious sulfide sols (63) raises a serious objection to the colorimetric pyroantimonate procedure of Yoshimatsu (98). The search for a colorimetric procedure for the determination of sodium is almost necessarily limited to the uranium triple salts.

With one exception, the colorimetric methods which have been developed for the determination of sodium by triple

Brown and Shohl (13), and Fritzen-Gerling (12).

8. The dihydroxyteric acid method of Fenton (28).

9. Spectrophotometric methods, as those of Thomson and Lee

(31) and Jansen, Heyes, and Blomster (32).

10. Methods using diamine cassein albits (3, 32).

11. Pyrocattonic methods, as those of Kramen (47).

Kramer and Glickman (48), and Lewin (33).

12. Uranium methods, as those of Blanchevère (9), Barber

and Koltzoff (6), and Wosiloff (49).

For routine clinical use, the following gravimetric analyses

are too tedious. Fenton's (28) method was gravimetric. Since

the spectrophotometric method is not a commonly used instrument in clinical

laboratories, a spectrophotometric method would have limited

application. Dole and Bell (22) in their development of the

diamine cassein albit method were unsuccessful in their

efforts to establish the method as a volumetric procedure.

Rowe's (29) modification of the Kramer and Glickman (48)

pyrocattonic method seems to be reproducible. But the in-

stability of uranicous albits (33) raises a serious

objection to the volumetric pyrocattonic procedure of

Yostmeyer (36). The search for a volumetric procedure for

the determination of sodium is almost necessarily limited to

the uranic triple salts.

With one exception, the volumetric methods which have

been developed for the determination of sodium by triple

acetate precipitation have depended on the measurement of the color of some compound of uranium. Woelfel (97) precipitated sodium as the uranyl manganese acetate, oxidized the manganese to permanganate, and measured its color. The presence of large quantities of organic matter in biological fluids and the ease of reduction of permanganate would seem to constitute an objection to the application of Woelfel's method to biological fluids, unless great precautions were taken to destroy all organic matter.

There is no reason for believing that a color reaction which works with the uranium in one of the triple acetate salts would not work equally well with that in any of the others. The choice of precipitant, therefore, was made on the basis of other properties of the individual triple salts. Caley (13) found that the cobalt salt is not suitable as a quantitative reagent for sodium because it does not give complete precipitation. Its suitability as a qualitative reagent depends on its lack of sensitivity to potassium. No results have been published on the use of nickel uranyl acetate as a quantitative reagent for sodium, and it was not studied here. The use of uranyl manganese acetate as the precipitant in a colorimetric method in which the color of a uranyl compound was measured would entail the complete suppression of color of the highly colored manganese compounds. Large errors have been found in the

gravimetric determination of sodium as the magnesium uranyl acetate by Kling and Lassieur (43) and by Barber and Kolthoff (6). Even Caley and Foulk (17), who have been the sole users of the magnesium triple salt in this country, admitted that the corresponding zinc salt gives better results with amounts of sodium less than 8 milligrams.

An examination of the literature on uranyl zinc acetate as a sodium reagent showed many advantages in its favor. In concentrated solution it quantitatively precipitates sodium as $(\text{UO}_2)_3 \text{ZnNa}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$ provided either that the reagent is first saturated with the sodium salt (6) or that the solubility of the sodium salt in the reagent is decreased by adding alcohol to the reagent (8). The sodium compound, the composition of which does not vary, can be separated from the excess of reagent easily (6). It is freely soluble in water. On the basis of these considerations uranyl zinc acetate was chosen as the precipitant.

Of the various color reactions of the uranyl ion outlined above, those with alizarin and cochineal were given little attention. The use of such colors would involve two major difficulties; first, the standardization of the cochineal or alizarin solutions, and second, the rigid control of acidity to prevent shifting of color characteristics of the excess^{of}/dye present in solution. Such difficulties represent unnecessary complications in a routine procedure.

gravimetric determination of sodium as the magnesium uranyl
acetate by Kline and Lassiter (23) and by Herber and Kofitoff
(24). Even today and Kofitoff (25), who have been the sole
users of the magnesium uranyl salt in this country, admitted
that the corresponding acid salt gives better results with
amounts of sodium less than 0.1 milligrams.

An examination of the literature on uranyl zinc acetate
as a sodium reagent showed many advantages in its favor.
In concentrated solution it quantitatively precipitates
sodium as $(UO_2)_2Zn(OAc)_6 \cdot 6H_2O$ provided either that the
reagent is first saturated with the sodium salt (26) or that
the solubility of the sodium salt in the reagent is decreased
by adding alcohol to the reagent (27). The sodium compound,
the composition of which does not vary, can be separated from
the excess of reagent easily (28). It is freely soluble in
water. On the basis of these considerations uranyl zinc
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Of the various color reactions of the uranyl ion out-
lined above, those with alizarin and cochineal were chosen
for this study. The use of each color would involve two
major difficulties: first, the standardization of the cochineal
or alizarin solutions, and second, the rigid control of
acidity to prevent shifting of color characteristics of the
excess dye present in solution. Such difficulties represent
unnecessary complications in a routine procedure.

The color of the triple salt itself has been studied by Hoffman and Osgood (36), who found that it is very unstable to heat and acidity. As they pointed out, the color can be stabilized by dissolving the triple salt in tenth-normal ammonium thiocyanate instead of in water. But ammonium thiocyanate is unstable in tenth-normal solution, and the use of this method entails the frequent standardization of the solvent.

The red color of uranyl ferrocyanide has been measured in the visual colorimeter in the methods of Barrenscheen and Messiner (8), Poulsson (74), McCance and Shipp (60), and Salit (82). As a preliminary step in the adaptation of this method to the photoelectric colorimeter, the absorption of light by the red compound was determined spectrophotometrically*. As shown by chart 1, the maximum absorption occurs somewhere in the ultraviolet spectrum. Measurement of light absorption by this substance in the Evelyn colorimeter is open to two objections: first, the colorimeter is not equipped to measure ultraviolet absorption, and by using this instrument one would measure the absorption of light which is not absorbed specifically by the compound; second, the blank is colored and also shows maximum absorption in the visible spectrum at 420 millimicra. Consequently, the ferrocyanide method was discarded.

*Quantitative measurements of light absorption (Charts 1, 2, and 3) were made on the recording spectrophotometer at the Color Measurement Laboratory of the Massachusetts Institute of Technology.

Chart 1. Light absorbed by compound of triple salt
with potassium ferrocyanide



Chart 2. Light absorbed by compound of triple salt with sodium salicylate

Preliminary studies of a series of phenolic and sulfhydryl compounds as colorimetric reagents for uranium showed that thioglycollic acid gives an intense red color with uranium. Unfortunately, it has a lingering, mephitic odor which discouraged further use. Tannic and gallic acids showed a strong tendency to form precipitates. Snell and Snell (86) have already mentioned this difficulty. Sodium salicylate and sulfosalicylic acid showed many advantages as colorimetric reagents. Both are very soluble in water, and both give colorless solutions when no uranium is present. Sulfosalicylic acid is a common reagent in clinical laboratories, and sodium salicylate is inexpensive enough to become one. The intensity of color formed by either reagent from the triple salt precipitated from .1 or .2 cc. of serum is such that the solution can be diluted to 100cc. to obtain galvanometer readings which fall in the most accurate part of the scale. Both colors follow Beer's law sufficiently well to allow application of the law over limited ranges of concentration. Neither color changed appreciably in intensity during three hours. The color with sodium salicylate showed a marked sensitivity to acetic acid and to sodium acetate. Sodium acetate is necessary for the development of color by sulfosalicylic acid. Spectrophotometric examination of the two colors (see Charts 2 and 3) showed that each has a narrow absorption band in the visible spectrum. With sodium

Chart 2. Light absorbed by compound of triple salt
with sodium salicylate



Chart 3. Light absorbed by compound of triple salt
with sulfosalicylic acid and sodium acetate



The following table shows the results of the experiments conducted on the 10th of May 1900. The experiments were conducted on the 10th of May 1900.



Graph showing the relationship between Time (minutes) and Temperature (degrees Celsius).

salicylate the maximum occurs at 465 millimicrons, with sulfosalicylic acid and sodium acetate, at 455 millimicrons. Since the salicylate color is so sensitive to acetates; since the absorption band of the sulfosalicylic acid color is more pronounced; and since it coincides more closely with a standard filter of the Evelyn colorimeter (maximum transmission, 440 millimicrons; range of transmission, 410-475 millimicrons), sulfosalicylic acid was chosen as the reagent for color development.

Development of method

In order to be able to estimate the errors in any particular step of the final method, the calibration curves for the color and the experiments in the development of the method were carried out on the basis of a standard solution prepared from uranyl acetate, zinc acetate, and sodium acetate, rather than on the basis of the triple salt precipitated from a standard solution of sodium chloride. The primary standard was prepared by dissolving in 500 cc. of water; 9.1308 gm. of uranyl acetate (Merck's "Reagent" $\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, lot 33008, assay 100.0%; 1.5745 gm. of zinc acetate (Merck's "Reagent" $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$; and 0.9763 gm. of sodium acetate (Merck's "Reagent" $\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$). This is equivalent to a solution containing 22.0706 gm. of $(\text{UO}_2)_3\text{ZnNa}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$ per liter; 1.0 cc. contains 0.330 gms. of sodium.

This artificial standard solution was chosen in preference to a standard solution of a weighed quantity of

salicylate the maximum occurs at 523 millimicrons, with salicylic acid and sodium acetate, at 428 millimicrons. Since the salicylate color is so sensitive to acetate; since the absorption band of the autooxidized acid color is more pronounced; and since it coincides more closely with a standard filter of the Evelyn colorimeter (maximum transmission, 440 millimicrons; range of transmission, 410-475 millimicrons), autooxidized acid was chosen as the reagent for color development.

Development of method

In order to be able to estimate the errors in any particular step of the final method, the calibration curves for the color and the absorption in the development of the method were carried out on the basis of a standard solution prepared from uranyl acetate, zinc acetate, and sodium acetate, rather than on the basis of the triple salt precipitated from a solution of sodium chloride. The primary standard was prepared by dissolving in 500 cc. of water; 2.1308 gm. of uranyl acetate (Merck's "Reagent" $UO_2(CH_3COO)_2 \cdot 2H_2O$, for 35002. assay 100.0%; 1.5745 gm. of zinc acetate (Merck's "Reagent" $Zn(CH_3COO)_2 \cdot 2H_2O$; and 0.9723 gm. of sodium acetate (Merck's "Reagent" $NaCH_3COO \cdot 3H_2O$). This is equivalent to a solution containing 25.0000 gm. of $UO_2(CH_3COO)_2 \cdot 2H_2O$ per liter; 1.0 cc. contains 0.350 kmo. of sodium.

This artificial standard solution was chosen in preference to a standard solution of a weighed quantity of

crystallized uranyl zinc sodium acetate for the following reasons. 1. The preparation of the triple salt by the method of Hoffman and Osgood (36) is wasteful of reagent, requiring a waste of uranium of over three times the weight of that recovered in the triple salt. 2. The method of McCance and Shipp (60) is less wasteful, yielding about 42% recovery, but a pure product could not be prepared by this method due to the reduction of uranium by the large amount of alcohol present.* A yellow precipitate was obtained, giving a color with sulfosalicylic acid 2.2% less intense than that of an equal concentration of the synthetic standard described above.

In the standardization of the color obtained from the triple salt with sulfosalicylic acid, two standard solutions were prepared and were diluted 1:20 with water. Varying amounts of these solutions were added to 100 cc. volumetric flasks and diluted to about 70 cc. with distilled water. Then were added in order ** 4 cc. of 5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and water to make 100 cc. The flasks were shaken and about 15 cc. of the contents transferred to Evelyn colorimeter tubes previously rinsed with the solution. The tubes were read in the colorimeter with filter 440 m μ and the values of K calculated from the known concentrations of sodium present and the observed galvanometer readings. The

* Lattimer and Hildebrand (52) mention this ease of reduction of uranyl salts by alcohol.

** Snell and Snell (86) state that a more intense color is obtained by adding the phenolic acid before the sodium acetate.

crystallized triethylamine acetate for the following reason. 1. The preparation of the triple salt by the method of Hoffman and Oseroff (56) is wasteful of reagent, resulting a waste of uranium of over three times the weight of that recovered in the triple salt. 2. The method of McLennan and Ship (60) is less wasteful, yielding about 45% recovery, but a pure product could not be prepared by this method due to the reduction of uranium by the large amount of alcohol present. A yellow precipitate was obtained, giving a color with sulfosalicylic acid 2.5% less intense than that of an equal concentration of the synthetic standard described above.

In the standardization of the color obtained from the triple salt with sulfosalicylic acid, two standard solutions were prepared and were diluted 1:20 with water. Varying amounts of these solutions were added to 100 cc. volumetric flasks and diluted to about 70 cc. with distilled water. Then were added in order 4 cc. of 0.5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and water to make 100 cc. The flasks were shaken and about 10 cc. of the contents transferred to Nessler colorimeter tubes previously rinsed with the solution. The tubes were read in the colorimeter with filter 440 m μ and the values of K calculated from the known concentrations of actinium present and the observed galvanometer readings. The

5. Lottner and Eliebrand (55) mention this case of reduction of triethylamine by alcohol.
56. Smith and Smith (58) state that a more intense color is obtained by adding the phenolic acid before the sodium acetate.

first four columns of tables 2 and 3 indicate the results of these experiments, and chart 4 shows the values of L plotted against the concentration of sodium in the sample. These tables indicate that the variations in the galvanometer readings for any particular concentration of sodium did not vary by more than one quarter of a scale division. By calculating $\Delta L/L$ as in table 1, it can be shown that the only instance in which this difference amounted to more than one percent was at the concentration of .132 mgms per 100cc., where the percentage difference is $\frac{.1163 - .1149}{.1163} \times 100$, or 1.2%.

Column 5 of tables 2 and 3 and chart 4 show that Beer's law does not apply exactly throughout the range of concentrations investigated. Instead of constant values of K in the tables and a straight-line plot of L against C in chart 4, the values of K show a constant downward trend with increasing concentration of color. The cause of this decrement of color intensity with increasing concentration was not investigated.

The decrease of K with increasing values of L was studied for the two standard solutions in the manner indicated by tables 4 and 5 and charts 5 and 6. Values of K , as calculated from the equation

$$K = L/C$$

were plotted against the corresponding values of L . The

First four columns of tables 2 and 3 indicate the results

of these experiments, and chart 4 shows the values of I plotted against the concentration of sodium in the sample.

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where the percentage difference is $\frac{.1163 - .1149}{.1163} \times 100$, or

1.22.

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Chart 4. Conformity with Beer's law of color
with sulfosalicylic acid and sodium acetate

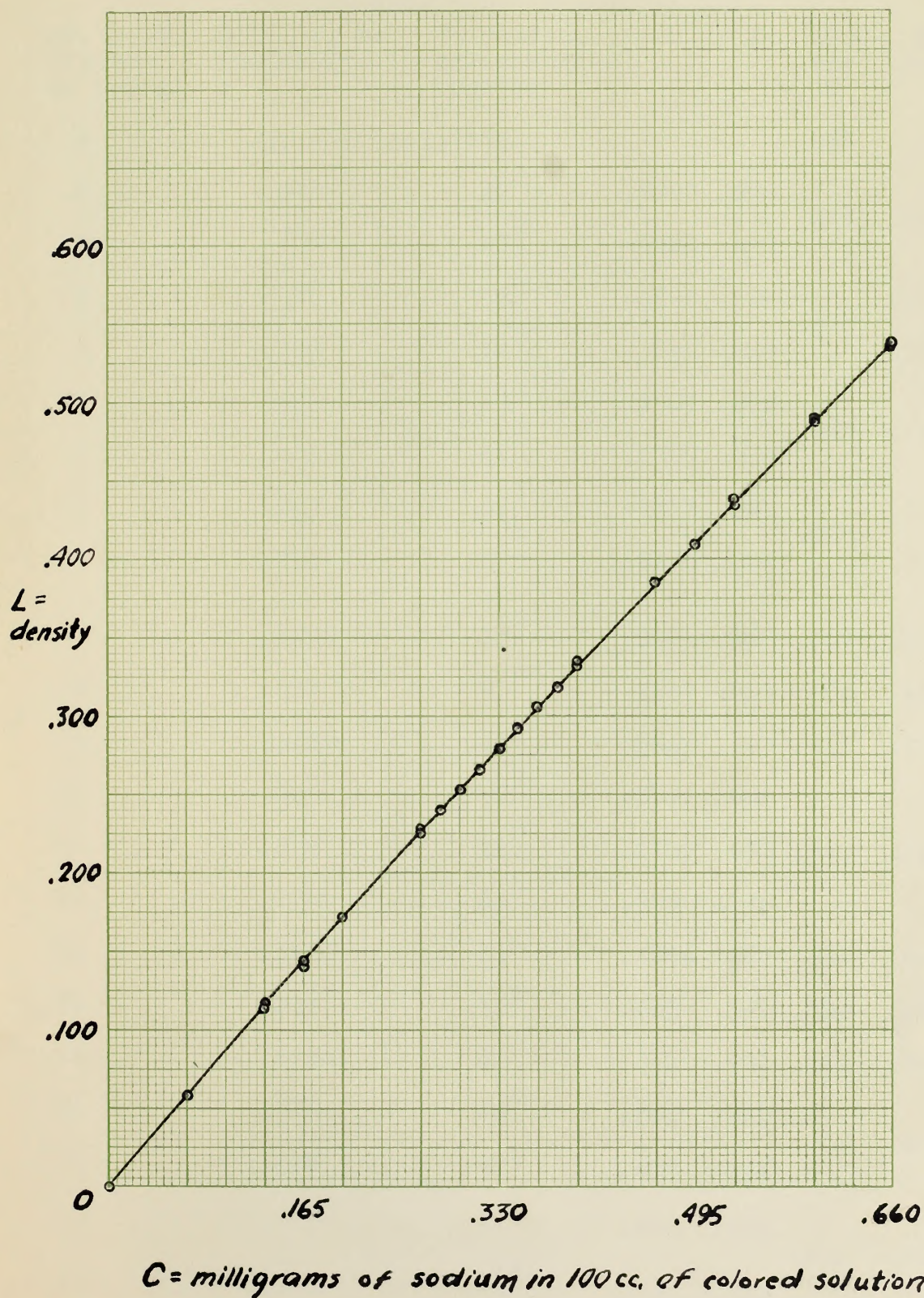


Table 2

cc. soln. diluted to 100cc.	Calibration of galvanometer					K	o/b calc. dif- ference
	Conc. of Na (mgm/100cc.)	Corrected galvanometer reading	L	K	Aver- age K		
0	By extrapolation				.889		
4	.066	87 ²	.0580	.879	.879	.881	-.2
4	.066	87 ²	.0580	.879			-.2
8	.132	76 ²	.1163	.881	.876	.872	+1.0
8	.132	76 ³	.1149	.870		.872	-.2
12	.198	67 ²	.1707	.862	.862	.864	-.2
12	.198	67 ²	.1707	.862			-.2
16	.264	59 ¹	.2273	.861	.858	.856	+.6
16	.264	59 ²	.2255	.854		.856	-.2
17	.2805	57 ²	.2403	.857	.857	.854	+.4
18	.297	55 ³	.2537	.854	.854	.852	+.2
19	.3135	54 ¹	.2656	.847	.847	.850	-.4
20	.330	52 ²	.2798	.848			-.1
20	.330	52 ²	.2798	.848	.848	.849	-.1
20	.330	52 ²	.2798	.848			-.1
21	.3465	51 ⁰	.2924	.844	.844	.847	-.4
22	.363	49 ²	.305	.840	.840	.845	-.6
23	.3795	48 ⁰	.319	.841	.841	.843	-.2
24	.396	46 ²	.332	.838			-.4
24	.396	46 ²	.332	.838	.838	.841	-.4
28	.462	41 ¹	.385	.833			-.1
28	.462	41 ¹			.833	.834	-.1
28	.462	41 ¹	.385	.833			-.1

Table 2

cc. soil diluted to 100cc.	Conc. of Na salvometer (mm/100cc.)	Corrected reading	Calibration of salvometer	Average K	K calc. for each sample
0		By extrapolation		.889	
4	.088	87 ^s	.0580 .879	.879	-.3
4	.088	87 ^s	.0580 .879	.881	-.3
8	.132	86 ^s	.1152 .881	.878	+1.0
8	.132	86 ^s	.1149 .870	.872	-.3
12	.198	87 ^s	.1707 .862	.862	-.3
12	.198	87 ^s	.1707 .862	.864	-.3
16	.264	89 ^l	.2272 .861	.863	+1.0
16	.264	89 ^s	.2253 .864	.868	-.3
17	.2805	87 ^s	.2402 .857	.854	+1.4
18	.297	85 ^s	.2337 .864	.858	+1.2
18	.2132	84 ^l	.2252 .847	.850	-.4
20	.330	82 ^s	.2798 .848		-.1
20	.330	82 ^s	.2798 .848	.848	-.1
20	.330	82 ^s	.2798 .848		-.1
21	.3463	81 ⁰	.2824 .844	.847	-.4
22	.362	48 ^s	.295 .840	.843	-.3
22	.3793	48 ⁰	.319 .841	.843	-.3
24	.398	45 ^s	.332 .838		-.4
24	.398	45 ^s	.332 .838	.841	-.4
28	.432	41 ^l	.383 .833	.833	-.1
28	.448	41 ^l	.383 .833	.834	-.1

Table 2 (cont.)

Calibration of galvanometer							
cc. soln. diluted to 100cc.	Conc. of Na (mgm/100cc)	Corrected galvano- meter reading	L	K	Aver- age K	K calc.	o/o dif- fer- ence
32	.528	36 ²	.438	.830	.827	.826	+ .5
32	.528	36 ³	.435	.824		.826	- .2
36	.594	32 ²	.488	.822	.819	.818	+ .5
36	.594	32 ³	.485	.816		.819	- .4
40	.660	29 ⁰	.538	.815	.812	.811	+ .5
40	.660	29 ¹	.534	.809		.812	- .4

Table 2
(cont.)

cc. soln. divided to 100cc.	Conc. of Na (mm/100cc)	Corrected galvano-meter reading	L Corrected	K	Average K	K calc. diff-fer-ence
32	.528	36	.428	.830	738.	.526 +.2
32	.528	36	.430	.824		.526 -.2
36	.524	32	.428	.832	818.	.518 +.2
36	.524	32	.428	.816		.518 -.4
40	.520	28	.428	.818	818.	.511 +.3
40	.520	28	.424	.808		.518 -.4

Table 3

cc. soln. diluted to 100cc.	Na mgm/100cc.	Reproducibility of color			K value of independently prepared solu- tion	% dif- ference of K values from	
		G	L	K		Obs.	Calc.
0	By extrapolation			.895	(ave.)	.889	
8	.132	{	76 ²	.1163.881		.872	+ .6 +1.0
			76 ²	.1163.881		.872	+ .6 +1.0
			ave.	.881	.876		
10	.165	{	71 ³	.1442.874		.868	+ .7
			71 ³	.1442.874		.868	+ .7
			72 ⁰	.1427.865		.868	-.3
			ave.	.871			
16	.264	{	59 ²	.2255.854		.856	-.5 -.2
			59 ¹	.2273.861		.856	+ .4 +.6
			ave.	.858	.858		
20	.330	{	52 ²	.2798.848		.849	.0 -.1
			52 ²	.2798.848		.849	.0 -.1
			52 ²	.2798.848		.849	.0 -.1
			ave.	.848	.848		
24	.396	{	46 ¹	.335 .846		.840	+ .9 +.7
			46 ²	.332 .838	.838	.841	.0 -.4
			ave.	.842			
30	.495	{	39 ⁰	.409 .826		.830	-.5
			39 ⁰	.409 .826		.830	-.5
			39 ⁰	.409 .826		.830	-.5
			ave.	.826			

Table 3 (cont.)

Reproducibility of color

cc. soln. diluted to 100cc.	Na mgm/100cc.	G	L	K	K value of independently prepared solu- tion	% differ- ence of K values from	
					Obs.	Calc.	Obs. Calc.
32	.528	{	36 ²	.438	.830		.826 +.4 +.5
			36 ³	.435	.824		.826 -.4 -.2
			ave.	.827	.827		
40	.660	{	29 ⁰	.538	.815		.811 +.4 +.5
			29 ¹	.534	.809		.812 -.4 -.4
			ave.	.812	.812		

Table 3 (cont.)

Reproducibility of color

No. soln. diluted to 100cc.		K value of independently prepared soln.		K value of soln. from Calc. Obs. Calc.	
32	328.	328.	328.	328.	328.
33	328.	328.	328.	328.	328.
34	328.	328.	328.	328.	328.
35	328.	328.	328.	328.	328.
36	328.	328.	328.	328.	328.
37	328.	328.	328.	328.	328.
38	328.	328.	328.	328.	328.
39	328.	328.	328.	328.	328.
40	328.	328.	328.	328.	328.
41	328.	328.	328.	328.	328.
42	328.	328.	328.	328.	328.
43	328.	328.	328.	328.	328.
44	328.	328.	328.	328.	328.
45	328.	328.	328.	328.	328.
46	328.	328.	328.	328.	328.
47	328.	328.	328.	328.	328.
48	328.	328.	328.	328.	328.
49	328.	328.	328.	328.	328.
50	328.	328.	328.	328.	328.
51	328.	328.	328.	328.	328.
52	328.	328.	328.	328.	328.
53	328.	328.	328.	328.	328.
54	328.	328.	328.	328.	328.
55	328.	328.	328.	328.	328.
56	328.	328.	328.	328.	328.
57	328.	328.	328.	328.	328.
58	328.	328.	328.	328.	328.
59	328.	328.	328.	328.	328.
60	328.	328.	328.	328.	328.
61	328.	328.	328.	328.	328.
62	328.	328.	328.	328.	328.
63	328.	328.	328.	328.	328.
64	328.	328.	328.	328.	328.
65	328.	328.	328.	328.	328.
66	328.	328.	328.	328.	328.
67	328.	328.	328.	328.	328.
68	328.	328.	328.	328.	328.
69	328.	328.	328.	328.	328.
70	328.	328.	328.	328.	328.
71	328.	328.	328.	328.	328.
72	328.	328.	328.	328.	328.
73	328.	328.	328.	328.	328.
74	328.	328.	328.	328.	328.
75	328.	328.	328.	328.	328.
76	328.	328.	328.	328.	328.
77	328.	328.	328.	328.	328.
78	328.	328.	328.	328.	328.
79	328.	328.	328.	328.	328.
80	328.	328.	328.	328.	328.
81	328.	328.	328.	328.	328.
82	328.	328.	328.	328.	328.
83	328.	328.	328.	328.	328.
84	328.	328.	328.	328.	328.
85	328.	328.	328.	328.	328.
86	328.	328.	328.	328.	328.
87	328.	328.	328.	328.	328.
88	328.	328.	328.	328.	328.
89	328.	328.	328.	328.	328.
90	328.	328.	328.	328.	328.
91	328.	328.	328.	328.	328.
92	328.	328.	328.	328.	328.
93	328.	328.	328.	328.	328.
94	328.	328.	328.	328.	328.
95	328.	328.	328.	328.	328.
96	328.	328.	328.	328.	328.
97	328.	328.	328.	328.	328.
98	328.	328.	328.	328.	328.
99	328.	328.	328.	328.	328.
100	328.	328.	328.	328.	328.

Chart 5. Variation of $\frac{\text{density}}{\text{concentration}}$ with density.

First solution

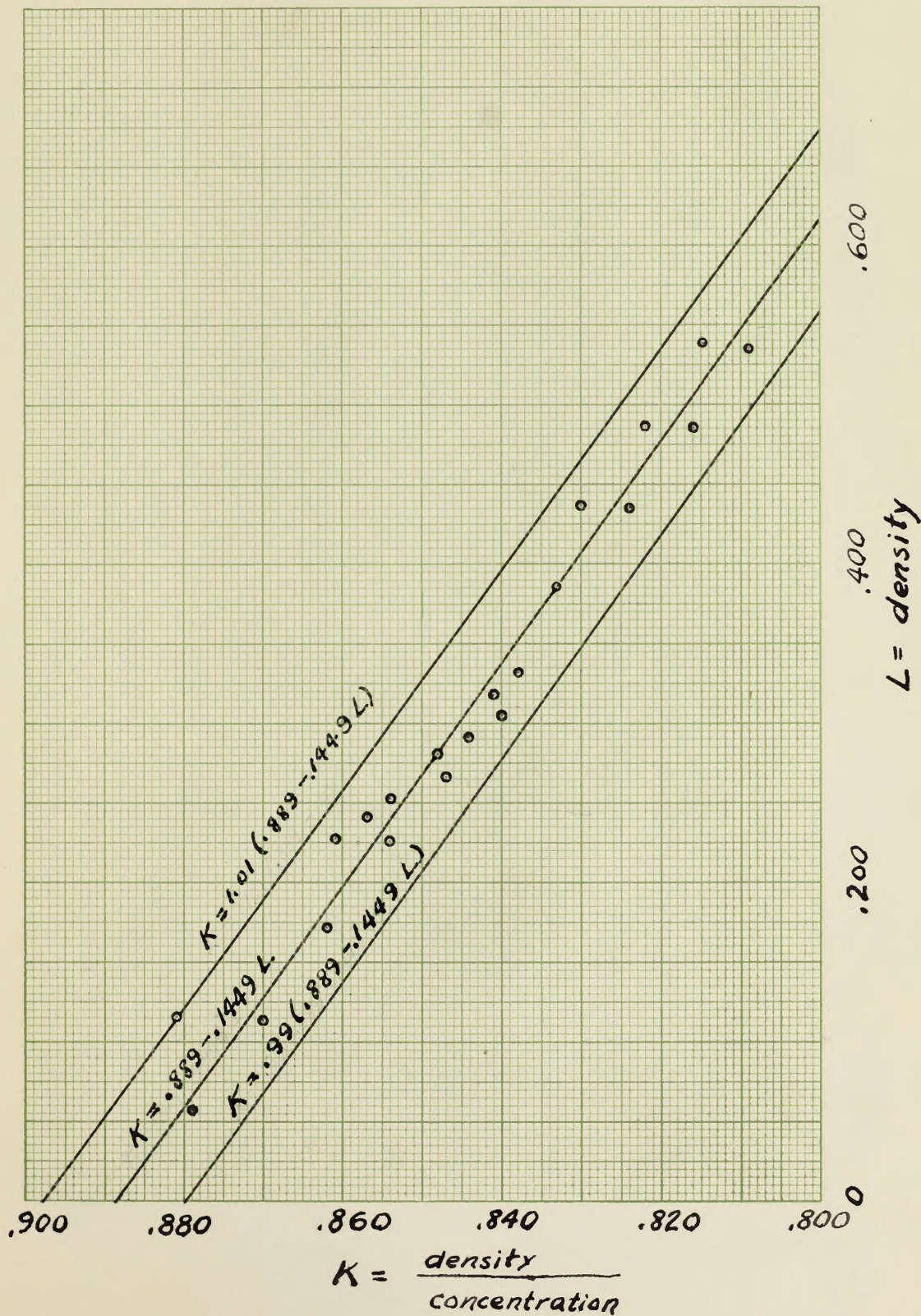


Table 4

Frequency	L	K	Variation of K_L	First solution	Calculations
2	.0580	.879	.05098	.00336	$27a + 8.0108b = 22.385$
2	.1156	.876	.10127	.01336	$8.0108a + 2.8498b = 6.7067$
2	.1707	.862	.14714	.02914	$27/8.0108 = 3.3704$
2	.2264	.858	.19425	.05126	$27a + 8.0108b = 22.835$
1	.2403	.857	.20594	.05774	$27a + 9.6050b = 22.604$
1	.2537	.854	.21666	.06436	$-1.5942b = .231$
1	.2656	.847	.22496	.07054	$b = -.1449$
3	.2798	.848	.23727	.07829	$27a - 8.0108x .1449 = 22.835$
1	.2924	.844	.24679	.08550	$27a - 1.161 = 22.835$
1	.305	.840	.25620	.09303	$27a = 23.996$
1	.319	.841	.26828	.10176	$a = .889$
2	.332	.838	.27822	.11022	Check
2	.385	.833	.32071	.14823	$8.0108x .889 - 2.8498x .1449 = 6.7067$
2	.437	.827	.36140	.19097	$7.1216 - .4129 = 6.7067$
2	.487	.819	.39885	.23717	$6.7087 = 6.7067$
2	.536	.812	.43523	.28730	Equation: $K = .889 - .1449L$

$\Sigma N = 27$ $\Sigma L = 8.0108$ $\Sigma K = 22.835$ $\Sigma LK = 6.70673$ $\Sigma L^2 = 2.84982$

Line: when $L = 0$, $K = .889$
 when $L = .500$,
 $K = .889 - .500x .1449$
 $= .889 - .07245$
 $= .817$

Chart 6. Variation of density with density.
concentration
Second solution

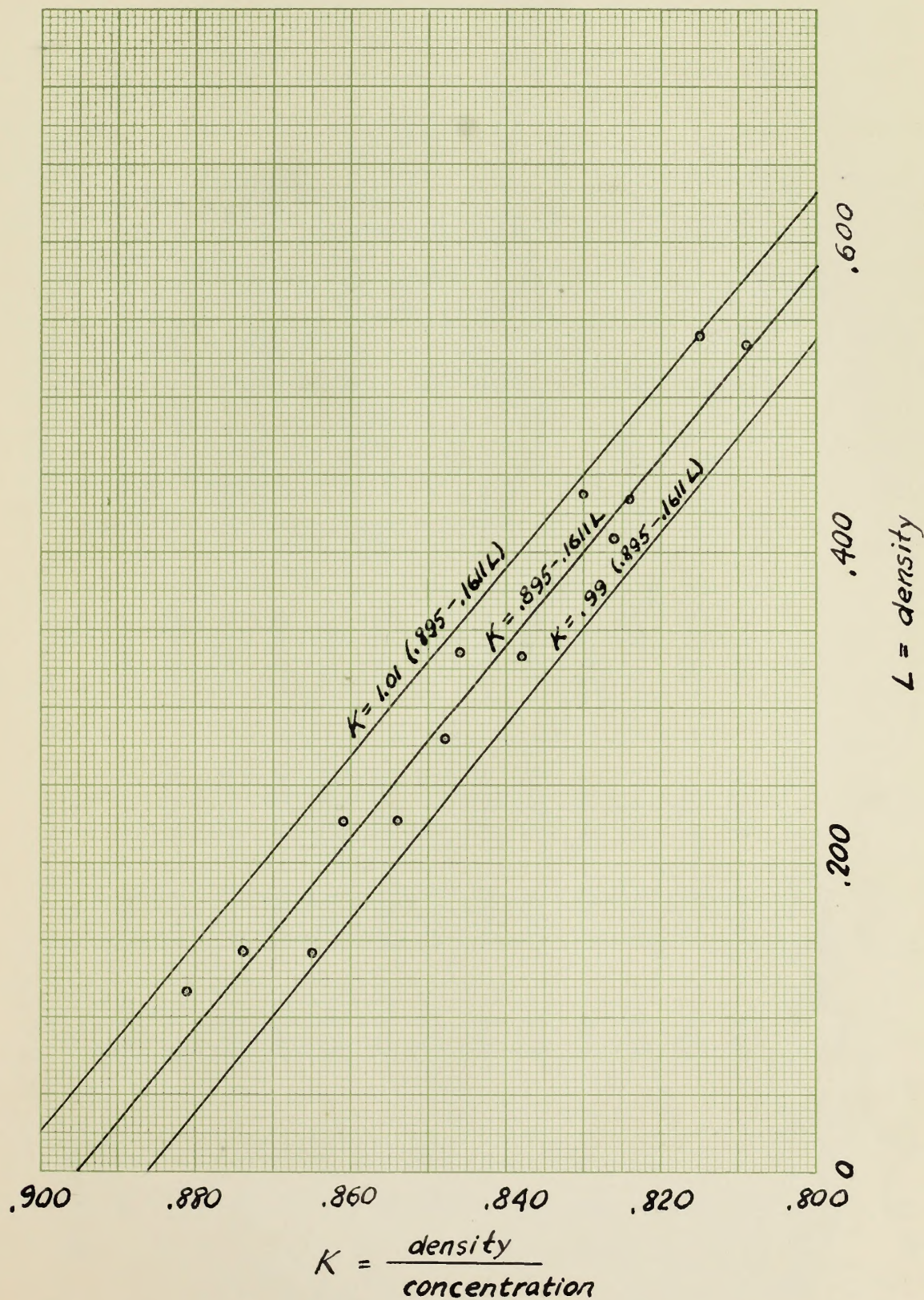


Table 5

Variation of K with L. Second solution

Calculations

Frequency

L

K

LK

L²

2

.1163

.881

.10246

.01353

19a + 5.7949b = 16.074

5.7949a + 2.10635b = 4.8481

19/5.7949 = 3.2787

1

.1427

.865

.12344

.02036

19a + 5.7949b = 16.074

19a + 6.9061b = 15.895

- 1.1112b = .179

b = -.1611

1

.2273

.861

.19571

.05167

19a - 5.7949 x .1611 = 16.074

19a - .934 = 16.074

19a = 17.008

a = .895

1

.332

.838

.27822

.11022

5.9749 x .895 - 2.10635 x .1611 = 4.8481

5.9749 x .895 - 2.10635 x .1611 = 4.8481

5.1864 - .3393 = 4.8471

EQUATION : K = .895 -.1611 L

LINE: when L = 0, K = .895

when L = .500, K = .895 - .500 x .1611

= .895 - .081

= .814

Σ n = 19

Σ L = 5.7949

Σ K = 16.074

Σ LK = 4.8481

Σ L² = 2.10635

equations for the values of K as functions of L were calculated for the two solutions by the method of least squares (70), and the lines corresponding to the derived equations were drawn in. As shown by the lines drawn in at values of K one percent greater or less than those calculated, these equations represent the observed data with a maximum inaccuracy of one percent. Columns 5,6,7, and 8 of table 2 show the actual percental difference between the observed and calculated values of K for one of the solutions.

Since the color does not follow Beer's law exactly, three methods are available for the calculation of the concentration of sodium in an unknown solution; ^{one}any of the three can be used to give results of the necessary accuracy.

(1) From the value of L derived from the galvanometer reading, the corresponding value of K can be read from chart 5, or can be calculated from the equation for the relationship of K to L. Then by dividing L by K, the figure for the concentration C is obtained.

(2) The value of C can be read directly from chart 4, the empirical curve drawn through the plot of observed values of L against the corresponding values of C.

(3) This method is based on the observed fact (column 5, tables 2 and 3), that over a range of $\pm 10\%$ in the value of L (and consequently the value of C), the value of K changes by only 1%. This statement holds true particularly well for concentrations of sodium where the corresponding

equations for the values of K as functions of L were calculated for the two solutions by the method of least squares (70), and the lines corresponding to the derived equations were drawn in. As shown by the lines drawn in at values of K one percent greater or less than those calculated, these equations represent the observed data with a maximum inaccuracy of one percent. Columns 2, 3, 4, and 5 of Table 2 show the actual percentage difference between the observed and calculated values of K for one of the solutions.

Since the color does not follow Beer's law exactly, three methods are available for the calculation of the concentration of sodium in an unknown solution; any of the three can be used to give results of the necessary accuracy.

(1) From the value of L derived from the colorimeter reading, the corresponding value of K can be read from chart 1, or can be calculated from the equation for the relationship of K to L . Then by dividing L by K , the figure for the concentration C is obtained.

(2) The value of C can be read directly from chart 4, the empirical curve drawn through the plot of observed values of L against the corresponding values of C .

(3) This method is based on the observed fact (column 2, Tables 2 and 3), that over a range of $\pm 10\%$ in the value of L and consequently the value of C , the value of K changes by only 1%. This statement holds true particularly well for concentrations of sodium where the corresponding

galvanometer readings fall near the middle of the scale. A deviation of $\pm 4\%$ in the sodium content of blood serum is clinically significant (36). A 2% error in the determination when the sodium content is 10% low would not be significant. The point in question is always whether or not the sodium level is significantly altered in comparison with the normal: if it is, the exact degree of alteration is relatively unimportant. In dealing with the sodium in blood, one may assume that Beer's law holds, and that

$$C = \frac{L}{K_n},$$

where C is the concentration of sodium in the blood, L is the density corresponding to the observed galvanometer reading, and K_n is the experimentally determined value of the ratio of L to C of a solution whose sodium content is equal to the mean normal value of blood sodium. Chart 7 shows the validity of these assumptions as applied in range of concentrations of .297 to .363 mgms.% of sodium, and that the errors within this range did not exceed 1%.

The dotted line on chart 7 was drawn on the assumption that within the limited range of concentrations indicated, the increase of concentration is directly proportional to the increase in density. This assumption was borne out experimentally, as shown by the close proximity of the experimental points to the line. The calculation^{*} of concentrations from observed densities by this relationship is too ponderous for everyday

*

Where C_H and C_L are the concentrations and L_H and L_L are the

calibrator readings fall near the middle of the scale. A

deviation of ± 0.5 in the sodium content of blood serum is clinically significant (36). A 2% error in the determination when the sodium content is 100 is not significant.

The point in question is always whether or not the sodium level is significantly altered in comparison with the normal.

It is, the exact degree of alteration is relatively unimportant. In dealing with the sodium in blood, one may assume that Beer's law holds, and that

$$D = \frac{L}{K} \cdot C$$

where C is the concentration of sodium in the blood, L is the density corresponding to the observed calibrator reading,

and K is the experimentally determined value of the ratio of

L to C of a solution whose sodium content is equal to the mean

normal value of blood sodium. Chart 7 shows the validity of

these assumptions as applied in range of concentrations of .297

to .363 mgm.% of sodium, and that the errors within this

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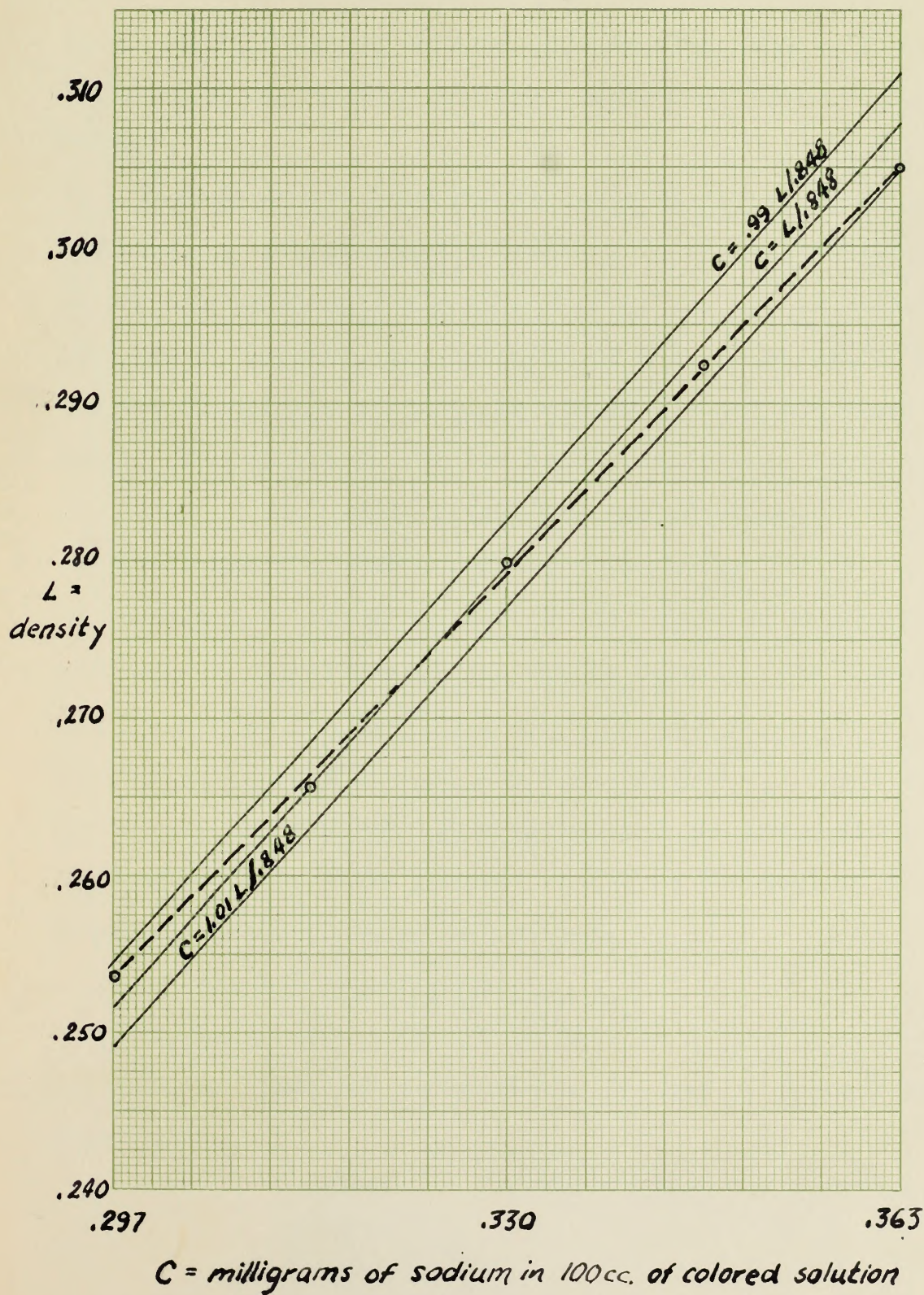
as shown by the close proximity of the experimental points to

the line. The calculation of concentrations from observed

densities by this relationship is too tedious for everyday

where C_1 and C_2 are the concentrations and L_1 and L_2 are the

Chart 7. Conformity with Beer's law over short range of concentration



work. The relationship is of value in connection with method 2, as outlined above, because it shows that the line on chart 4 can be drawn in small straight segments without serious error.

The reproducibility of the color is indicated by the duplication of galvanometer readings of equivalent dilutions of the two solutions, shown in tables 2 and 3. As a check on the reproducibility, the relation of K to L was studied for the two solutions, as shown in tables 4 and 5 and charts 5 and 6. The equations for the relationship were found to be

$$K = .889 - .1449L \text{ for one solution}$$

$$\text{and } K = .895 - .1611L \text{ for the other.}$$

In columns 5, 6, and 8 of table 3 the values of K observed for varying concentrations of the second solution are compared

corresponding densities of the high and low extremes, respectively, over which the principle of the proportionality of increments is to be applied, and L is the density whose concentration C is to be determined,

$$C = C_L + \frac{(L - L_L)}{(L_H - L_L) / (C_H - C_L)} .$$

For example, from the experimental figures in table 2 and chart 7,

$$C_{.2924} = .297 + \frac{(.2927 - .2537)}{(.305 - .2537) / (.363 - .297)}$$

$$C_{\text{calc.}} = .347 .$$

$$\text{Concentration taken} = .3465 .$$

work. The relationship is of value in connection with method 3, as outlined above, because it shows that the line on graph 4 can be drawn in small straight segments without serious error.

The reproducibility of the color is indicated by the duplication of galvanometer readings of equivalent dilutions of the two solutions, shown in tables 4 and 5. As a check on the reproducibility, the relation of K to L was studied for the two solutions, as shown in tables 4 and 5 and graphs 3 and 6. The equations for the relationship were found to be

$$K = .895 - .144L \text{ for one solution}$$

$$\text{and } K = .895 - .141L \text{ for the other.}$$

In columns 3, 6, and 8 of table 5 the values of K observed for varying concentrations of the second solution are compared

correcting factors of the high and low extremes, respectively, over which the principle of the proportionality of increments is to be applied, and L is the density whose concentration C is to be determined.

$$C = \frac{C_L + (C_H - C_L)}{(D_H - D_L) \sqrt{(D_H - D_L)}}$$

For example, from the experimental figures in table 5 and

$$C_{\text{calc}} = .237 + \frac{(.237 - .237)}{(.237 - .237) \sqrt{(.237 - .237)}}$$

$$C_{\text{calc}} = .237$$

Concentration taken = .237

with the average of observed K values for corresponding concentrations of the first solution. The limits of variation were found to be from $-.5\%$ to $+.9\%$, the average variation $\pm .33\%$. In columns 5, 7, and 9 of table 3, the values of K observed for dilutions of the second solution are compared with the values expected from the observed values of L and the K-L regression equation of the first solution. The limits of variation were found to be from $-.5\%$ to 1.0% , the average variation $\pm .48\%$.

In the study of the stability of the color with respect to time, readings were made at intervals on tubes containing about 15 cc. of varying dilutions of the triple salt, made up in every case with 4 cc. of 5% sulfosalicylic acid and 4 cc. of 10% sodium acetate per 100cc. The liquids were left in the tubes between readings, and the standardized tubes were not used throughout; consequently, the readings for any particular concentration do not necessarily check either with one another or with the readings in tables 2 and 3. Results are shown in table 6.

No changes in color were found within the first three hours, but decreases of galvanometer readings implying increases of color corresponding to about 1% were observed in most tubes after ten hours.

Table 7 shows the results of experiments testing the effect of temperature on the color of the solution. Solutions containing the indicated concentration of triple salt

with the average of observed K values for corresponding concentrations of the first solution. The limits of variation were found to be from -0.5% to $+0.5\%$, the average variation $\pm 0.3\%$. In columns 5, 7, and 8 of table 3, the values of K observed for dilutions of the second solution are compared with the values expected from the observed values of L and the K-L regression equation of the first solution. The limits of variation were found to be from -0.5% to $+1.0\%$, the average variation $\pm 0.4\%$.

In the study of the stability of the color with respect to time, readings were made at intervals on tubes containing about 10 cc. of varying dilutions of the triple salt, made up in every case with 4 cc. of 5% sulfosalicylic acid and 4 cc. of 10% sodium acetate per 100 cc. The readings were first in the tubes between readings, and the standardized tubes were not used throughout; consequently, the readings for any particular concentration do not necessarily check either with one another or with the readings in tables 2 and 3. Results are shown in table 6.

No changes in color were found within the first three hours, but decreases of galvanometer readings implying increases of color corresponding to about 1% were observed in most tubes after ten hours.

Table 7 shows the results of experiments testing the effect of temperature on the color of the solution. Solutions containing the indicated concentration of triple salt

Table 6

Permanence of the color

Na conc. (mgms.%)	Corrected Galvanometer readings immediately	after 1 hour	after 3 hours	after 10 hours
.033	93 ¹	93 ¹		93 ¹
.066	87 ¹	87 ¹		87 ¹
.165	71 ³	71 ³		71 ³
	71 ³		71 ³	
	71 ³		71 ³	
	72 ⁰		72 ⁰	
	71 ³			71 ³
	71 ³			71 ³
	72 ⁰			72 ⁰
.330	52 ²	52 ²		52 ¹
	52 ²		52 ²	
	52 ²		52 ²	
	52 ²		52 ²	
	52 ²			52 ¹
	52 ²			52 ¹
	52 ²			52 ¹
.495	39 ¹	39 ¹		39 ⁰
	39 ⁰		39 ⁰	
	39 ⁰		39 ⁰	
	39 ⁰		39 ⁰	
	39 ⁰			38 ³

Table 8

Persistence of the color

We conc. (mm. %)	Corrected Galvanometer readings immediately	after 1 hour	after 3 hours	after 10 hours
.033	93 ₁	93 ₁		93 ₁
.066	87 ₁	87 ₁		87 ₁
.165	71 ₂	71 ₂		71 ₂
	71 ₂		71 ₂	
	71 ₂		71 ₂	
	72 ₀		72 ₀	
	71 ₂			71 ₂
	71 ₂			71 ₂
	72 ₀			72 ₀
.330	82 ₂	82 ₂		82 ₁
	82 ₂		82 ₂	
	82 ₂		82 ₂	
	82 ₂		82 ₂	
	82 ₂		82 ₂	
	82 ₂			82 ₁
	82 ₂			82 ₁
	82 ₂			82 ₁
.465	93 ₁	93 ₁		93 ₁
	93 ₀		93 ₀	
	93 ₀		93 ₀	
	93 ₀		93 ₀	
	93 ₀			93 ₀
	93 ₀			93 ₀

Table 6 (Cont.)

Permanence of the color

Na conc. (mgms.%)	Corrected Galvanometer readings			
	immediately	after 1 hour	after 3 hours	after 10 hours
.495	39 ⁰			38 ³
	38 ³			38 ²
.660	29 ³	29 ³		29 ²

Table 7

Effect of temperature on the color

Na mgms.%	Corrected galvanometer readings at			
	17.5° C.	20.5° C.	25.3° C.	30.5° C.
.165	72 ⁰	71 ³	71 ³	71 ³
	72 ¹	72 ¹	72 ⁰	72 ⁰
	72 ⁰	72 ⁰	71 ³	71 ³
.330	52 ³	52 ³	52 ²	52 ²
	53 ⁰	53 ⁰	52 ²	52 ²
	52 ³	52 ³	52 ¹	52 ²
.495	39 ¹	39 ¹	39 ⁰	38 ³
	39 ¹	39 ¹	39 ⁰	39 ⁰
	39 ¹	39 ¹	39 ⁰	39 ⁰

Table 6 (Cont.)

Persistence of the color

Na conc. (mmole/l.)	Corrected galvanometer readings immediately	after 1 hour	after 3 hours	after 10 hours
4.35	38.0			38.3
	38.3			38.3
6.60	38.3	39.3		39.3

Table 7

Effect of temperature on the color

Na mmole/l.	17.5°C.	20.0°C.	25.0°C.	30.0°C.
1.65	72.0	71.8	71.8	71.5
	72.1	72.1	72.0	72.0
	72.0	72.0	71.8	71.5
3.30	32.3	32.3	32.3	32.3
	32.0	32.0	32.3	32.3
	32.3	32.3	32.1	32.3
4.95	32.1	32.1	32.0	32.3
	32.1	32.1	32.0	32.0
	32.1	32.1	32.0	32.0

plus 4 cc. of each of 5% sulfosalicylic acid and 10% sodium acetate were prepared in 100 cc. volumetric flasks. The flasks were placed in a water bath at the temperature indicated for 20 minutes. About 15 cc. of the contents were transferred to Evelyn tubes rinsed with the solution, and the tubes were immediately replaced in the water bath. The outside of the tubes were dried and the tubes read in the colorimeter. Immediately after reading the temperature of the solution in each tube was taken. The results in table 7 indicate a barely detectable decrease in intensity (.7-1.0%) when the temperature is 20° or below from that observed at 25° and above. As the room temperature varied from 24° to 26.5° during the course of the experiments reported in this paper, this temperature effect was neglected. If the room temperature varied markedly or fell below 23°, it would, of course, be necessary to apply a correction factor of plus one quarter scale division to those readings from 72 to 36, more or less, taken below this temperature.

Hoffman and Osgood (36) reported that the effect of temperature on the color of a pure solution of the triple salt is so marked that the heat of the colorimeter lamp affects it. No such sensitivity was detected in the color developed from the triple salt by sulfosalicylic acid. Three tubes gave identical galvanometer readings before and after being exposed to the heat of the lamp for two minutes-more

plus 4 cc. of each of 3% sulfosalicylic acid and 10% sodium acetate were prepared in 100 cc. volumetric flasks. The flasks were placed in a water bath at the temperature indicated for 30 minutes. About 15 cc. of the contents were transferred to Evelyn tubes rinsed with the solution, and the tubes were immediately replaced in the water bath. The outside of the tubes were dried and the tubes read in the colorimeter. Immediately after reading the temperature of the solution in each tube was taken. The results in Table V indicate a barely detectable decrease in intensity (.7-1.0%) when the temperature is 20° or below from that observed at 25° and above. As the room temperature varied from 24° to 26.5° during the course of the experiments reported in this paper, this temperature effect was neglected. If the room temperature varied markedly or fell below 25°, it would, of course, be necessary to apply a correction factor of plus one greater scale division to those readings from 25 to 26, more or less, taken below this temperature.

Hoffman and Garrod (3a) reported that the effect of temperature on the color of a pure solution of the triple salt is so marked that the heat of the colorimeter lamp affects it. No such sensitivity was detected in the color developed from the triple salt by sulfosalicylic acid. Three tubes gave identical photometer readings before and after being exposed to the heat of the lamp for two minutes more

than 4 times the normal length of time required for making a reading in the photoelectric colorimeter.

The color developed by sulfosalicylic acid was found to be quite sensitive to changes of acidity. The addition of mineral acid diminished the color markedly. The dropwise addition of sodium hydroxide increased the color to a density about twice as great as that used in these experiments; further additions caused rapid fading of the color. The most intense color could be obtained by neutralizing the sulfosalicylic acid to a phenolphthalein end point before adding it to the uranyl solution. The pH of the colored solutions with sulfosalicylic acid and sodium acetate varied from 4.5 to 5.3 with increasing concentrations of uranium; the pH of solutions with neutralized sulfosalicylic acid varied from 9.1 to 5.5 with increasing concentrations of uranium. The more intense color was not used in further experiments because it was found to be much more sensitive to changes of acidity than was the color with sulfosalicylic acid and sodium acetate, and because it did not follow Beer's law nearly as well as did the original color. The density of the original color increased 94% between concentrations of sodium of .165 mgms. per 100 cc. and .330 mgms. per 100 cc.; over the same range, the density of the color with neutralized sulfosalicylic acid increased only 69%.

FARSONS

FALCON BOND

RAW CONTENT

An experiment was performed to determine the effect of changing the relative and absolute amounts of sodium acetate and sulfosalicylic acid. To each of twelve 100 cc. volumetric flasks was added 20 cc. of triple salt solution containing .330 mgms. of sodium. Varying amounts of sulfosalicylic acid and sodium acetate were added, and the contents diluted to volume and read in the colorimeter. Results are shown in table 8.

Increasing the amounts of sodium acetate and sulfosalicylic acid had no effect on the color as long as the ratio of the two reagents was not varied. Increasing the ratio of sodium acetate to sulfosalicylic acid caused a decrease of intensity.

On the basis of these results, precautions were taken to measure accurately the color reagents and to make subsequent solutions of sulfosalicylic acid of equal concentration to that of the solution used in standardization (5 cc. of sulfosalicylic acid solution required 17.25 cc. 0.1000 N NaOH to neutralize it to a phenolphthalein end point).

Because of the solubility of uranyl zinc sodium acetate in water, it is necessary to take special precautions to insure completeness of precipitation of the sodium in any specimen. The precipitating reagent is made very concentrated in uranyl zinc acetate, but the volume of the sample solution reduces this somewhat. Two general methods have been proposed for insuring more complete precipitation. In their

An experiment was performed to determine the effect of changing the relative and absolute amounts of sodium acetate and sulfosalicylic acid. To each of twelve 100 cc. volumetric flasks was added 20 cc. of triple salt solution containing .350 mmoles of sodium. Varying amounts of sulfosalicylic acid and sodium acetate were added, and the contents diluted to volume and read in the colorimeter. Results are shown in Table 8.

Increasing the amount of sodium acetate and sulfosalicylic acid had no effect on the color as long as the ratio of the two reagents was not varied. Increasing the ratio of sodium acetate to sulfosalicylic acid caused a decrease of intensity.

On the basis of these results, precautions were taken to measure accurately the color reactions and to make subsequent solutions of sulfosalicylic acid of equal concentration to that of the solution used in standardization (5 cc. of sulfosalicylic acid solution per 10 cc. of 0.1000 N NaOH to precipitate it to a phenolphthalein end point).

Because of the solubility of uranyl zinc sodium acetate in water, it is necessary to take special precautions to insure completeness of precipitation of the sodium in any specimen. The precipitation reagent is made very concentrated in uranyl zinc acetate, but the volume of the sample solution required is somewhat. Two general methods have been proposed for insuring more complete precipitation. In their

Table 8

Effect of varying amounts of reagents on color density.

Na Mgms. %	5% Sulfosalicylic acid added cc.	10% NaAc added cc.	Corrected galvanometer reading	Density
0	4.0	4.0	Central setting 73 ³	
	4.0	4.0	52 ²	.2798
	4.0	4.0	52 ²	.2798
	3.7	4.0	52 ³	.2777
	3.7	4.0	52 ³	.2777
.330	3.7	4.3	53 ¹	.2736
	3.7	4.3	53 ¹	.2736
	3.7	3.7	52 ²	.2798
	3.7	3.7	52 ²	.2798
	4.0	3.7	51 ²	.2882
	4.0	3.7	51 ³	.2861
	4.3	4.3	52 ²	.2798
	4.3	4.3	52 ²	.2798

Table 3

Effect of varying amounts of reagents on color density.

Na Moms. 4	0.5 Sulfonil- cyllic acid added	10% Wash added	Corrected Galvanometer reading	Density
0	4.0	4.0	Central setting 73	
	4.0	4.0	52	.2798
	4.0	4.0	52	.2798
	3.7	4.0	52	.2777
	3.7	4.0	52	.2777
330	3.7	4.3	51	.2756
	3.7	4.3	51	.2736
	3.7	3.7	52	.2746
	3.7	3.7	52	.2748
	4.0	3.7	51	.2662
	4.0	3.7	51	.2661
	4.3	4.3	52	.2738
	4.3	4.3	52	.2738

original procedure, Barber and Kolthoff (6) saturated the reagent with the triple salt and filtered immediately before using the reagent. Butler and Tuthill (12), Ball and Sadusk (4), and Hoffman and Osgood (36) used this method. Barrenscheen and Messiner (8) did not saturate the reagent with the triple salt, but used alcohol in the precipitating mixture. McCance and Shipp (60), Snell and Snell (86), Salit (82), Jendrassik and Dziobek (39), Ruzsnyák and Hatz (81), Weinbach (96), Chen (19), and Marenzi and Gerschmann (55,56) used alcohol in amounts varying from 11% to 50% of the total volume of sample solution plus precipitants.

Holmes and Kirk (37), using the saturated precipitant, found it to be necessary to carry out the precipitation in a refrigerator to insure constancy of values, although Ball and Sadusk (4) worked at room temperature. Hoffman and Osgood (36) pointed out that precipitation is not absolutely complete with the saturated reagent because of the dilution of the reagent by the sample solution.

Regarding the use of alcohol, it has already been pointed out (p. 26) that uranyl salts are unstable in the presence of alcohol. Thus it is necessary to add the alcohol at the time of precipitation. Snell and Snell (86) reported 33% as the optimal concentration of alcohol in the precipitating mixture, but Marenzi and Gerschmann (56) stated that 25% is the highest concentration which can be used without precipitating some of the uranyl zinc acetate. The procedure of

original procedure, Barber and Kellhoff (3) saturated the reagent with the triple salt and filtered immediately before using the reagent. Butler and Tschiff (12), Ball and Badask (4), and Hoffman and Gassod (38) used this method. Garren-achsen and Messner (8) did not saturate the reagent with the triple salt, but used alcohol in the precipitation mixture. McCance and Shipp (50), Small and Small (52), Salit (53), Landrassik and Doleak (54), Rasmussen and Hata (51), Weinbach (55), Chen (19), and Merenz and Gerschmann (56,57) used alcohol in amounts varying from 1% to 5% of the total volume of sample solution plus precipitant. Holmes and Kirk (57), using the saturated precipitant, found it to be necessary to carry out the precipitation in a refrigerator to insure constancy of values, although Ball and Badask (4) worked at room temperature. Hoffman and Gassod (38) pointed out that precipitation is not absolutely complete with the saturated reagent because of the dilution of the reagent by the sample solution. Regarding the use of alcohol, it has already been pointed out (p. 36) that triple salts are unstable in the presence of alcohol. Thus it is necessary to add the alcohol at the time of precipitation. Small and Small (56) reported 3% as the optimal concentration of alcohol in the precipitating mixture, but Merenz and Gerschmann (56) stated that 5% is the highest concentration which can be used without precipitating some of the uranyl zinc acetate. The procedure of

Weinbach (96), in which 2.1 cc. of 95% alcohol is added in small portions to 1 cc. of sample and 5 cc. of reagent, was used in the experiments summarized in table 9.

The triple acetate precipitate must be washed free from adherent reagent before it can be determined accurately by any of the procedures so far devised. Barber and Kolthoff (6) and Butler and Tuthill (12) used a saturated alcoholic solution of uranyl zinc sodium acetate for washing out the excess of reagent. Hald (35), Salit (82), and Ball and Sadusk (4) found this reagent to be unsatisfactory because of the decomposition of the uranium in the solution. Salit (82) introduced the use of a saturated solution of triple salt in glacial acetic acid for the first washing; he then washed out the acetic acid with ether. Marenzi and Gerschmann (55,56), Chen (19), and Ball and Sadusk (4) found these to be satisfactory wash reagents. Weinbach (96) washed the precipitate once with a saturated solution of the triple salt in acetone. In attempting to use this reagent in the experiments here reported, it was found that the galvanometer readings obtained were much lower than the theoretical values for standard triple salt solutions. This would indicate that the wash reagent precipitates, rather than dissolves, the excess of reagent. By mixing the wash liquid with the precipitant, this surmise was verified.

Hoffman and Osgood (36) found that all three of the wash reagents mentioned produced precipitates of triple salt when

Weinbach (56), in which 2.1 cc. of 5% alcohol is added in small portions to 1 cc. of sample and 5 cc. of reagent, was used in the experiments summarized in Table 2.

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Hoffman and Osgood (38) found that all three of the wash reagents mentioned produced precipitates of triple salt when

brought in contact with the precipitant, and that the acetic acid reagent produced the least precipitate. They also observed that the pure liquids produced no precipitate. Because of the irritating odor of glacial acetic acid, they used a 15% solution of acetic acid in alcohol, saturated with the triple salt.

By decreasing the solubility of uranyl zinc sodium acetate in acetic acid with the addition of ethyl acetate, it was possible in these experiments to avoid the use of saturated wash liquids and to evade the obvious limitations of such reagents. About 10 mgm. samples of uranyl zinc sodium acetate were shaken with 5 cc. each of ethyl alcohol, 15% acetic acid in alcohol, glacial acetic acid, 10% ethyl acetate in acetic acid, and ether. After allowing the excess of triple salt to settle, a few drops of the supernatant fluids were mixed with an equal volume of potassium ferrocyanide solution. The solubility of the triple salt in the liquids, as estimated by the intensity of red color produced with ferrocyanide, was found to decrease in the order above mentioned.

Acetic acid solutions containing 0, 20, 40, 60, and 80% by volume of ethyl acetate were shaken with equal volumes of uranyl zinc acetate reagent (96) and with 10 mgm. samples of the triple salt. Glacial acetic acid and the 20% ethyl acetate solution gave no precipitate with the reagent. The 40% solution produced a slight precipitate in $\frac{1}{2}$ hour, the 60%

brought in contact with the precipitate, and that the acetic acid reagent produced the least precipitate. They also observed that the pure lipids produced no precipitate. The cause of the irritating odor of glacial acetic acid, they used a 10% solution of acetic acid in alcohol, saturated with the triple salt.

By decreasing the solubility of uranyl zinc acetate in acetic acid with the addition of ethyl acetate, it was possible in these experiments to avoid the use of saturated wash liquids and to evade the obvious limitations of such reagents. About 10 mm. samples of uranyl zinc acetate were shaken with 3 cc. each of ethyl alcohol, 10% acetic acid in alcohol, glacial acetic acid, 10% ethyl acetate in acetic acid, and ether. After allowing the excess of triple salt to settle, a few drops of the supernatant liquids were mixed with an equal volume of potassium ferrioxalate solution. The solubility of the triple salt in the liquids, as estimated by the intensity of red color produced with ferrioxalate, was found to decrease in the order above mentioned.

Acetic acid solutions containing 0, 20, 40, 60, and 80% by volume of ethyl acetate were shaken with equal volumes of uranyl zinc acetate reagent (5%) and with 10 mm. samples of the triple salt. Glacial acetic acid and the 20% ethyl acetate solution gave no precipitate with the reagent. The 40% solution produced a slight precipitate in 1 hour, the 60%

solution immediately. The mixture with the 80% solution separated into a solid and 2 liquid phases. The supernatant fluids from the triple salt were tested with ferrocyanide as before. The solutions containing 40% or more ethyl acetate gave no color.

A solution containing 30% of ethyl acetate in acetic acid was found to give no color with ferrocyanide after shaking with triple salt, and to give no precipitate with an equal volume of uranyl zinc acetate reagent. Although this solution has a very irritating odor, it can be used without discomfort by delivering it from a wash bottle if a third hole, containing a short glass tube, is put through the stopper and a Bunsen valve attached to the inner end of the mouthpiece. The flow of liquid is regulated by placing the index finger over the third tubing.

The efficiency of precipitation and washing was tested by analyzing standard solutions of sodium chloride. Varying amounts of standard solution of sodium chloride were delivered into centrifuge tubes from calibrated intervals of a microburette and diluted to 1 cc.; 5 cc. of uranyl zinc acetate reagent, prepared according to Weinbach⁽⁹⁶⁾, were added, followed by seven .3cc portions of 95% ethyl alcohol during $\frac{1}{2}$ hour. The tubes were centrifuged for 10 minutes at 2000 r.p.m., decanted and inverted over a piece of cheesecloth for 5 minutes. The mouths of the tubes were then wiped dry, and into each was blown about 1 cc. of 30% ethyl acetate in

solution immediately. The mixture with the 50% solution separated into a solid and 2 liquid phases. The supernatant liquids from the triple salt were tested with ferricyanide as before. The solutions containing 40% or more ethyl acetate gave no color.

A solution containing 30% of ethyl acetate in acetone

solid was found to give no color with ferricyanide after shaking with triple salt, and to give no precipitate with an equal volume of acetyl zinc acetate reagent. Although this solution has a very irritating odor, it can be used without discomfort by delivering it from a wash bottle in a

third role, containing a short glass tube, is put through the stopper and a T-shaped valve attached to the inner end of the manometer. The flow of liquid is regulated by placing the index finger over the third tubing.

The efficiency of precipitation and washing was tested by analyzing standard solutions of sodium chloride. Various amounts of standard solution of sodium chloride were de-

livered into centrifuge tubes from calibrated intervals of a graduated cylinder and diluted to 1 cc. of acetyl zinc

acetate reagent, prepared according to Weidbach, were added, followed by seven .5cc portions of 95% ethyl alcohol during

4 hours. The tubes were centrifuged for 10 minutes at 2000

r.p.m., decanted and inverted over a piece of cheesecloth

for 5 minutes. The contents of the tubes were then wiped dry,

and into each was blown about 1 cc. of 50% ethyl acetate in

acetic acid. The precipitate was agitated by holding the tube firmly between the thumb and index finger of the left hand and tapping the base with the right forefinger, thus producing a rotatory motion of the liquid in the tube. The walls of the tubes were then washed down with about 2 cc. more of liquid. The tubes were again centrifuged for 10 minutes, decanted, inverted for five minutes, and wiped as before. The precipitate was then washed twice with 5 cc. portions of ether to remove the acetic acid, which interferes with the color with sulfosalicylic acid. After ether washings the tubes were centrifuged for only 5 minutes and drained for only one minute. Longer draining after ether washings may allow the precipitate to become so dry that it falls out of the tube. After draining from the second ether washing, the tubes were placed over a steam radiator for 5 minutes to dry. The contents of each tube were dissolved by agitating with 3-4 cc. of water, transferred quantitatively to a 100 cc. volumetric flask, and diluted to about 70 cc. Then were added 4 cc. of 5% sulfosalicylic acid solution and 4 cc. of 10% acetic acid solution, and the solutions diluted to volume and mixed. A 15 cc. portion of the solution in each flask was transferred to an Evelyn tube previously rinsed with the solution, and the tubes were read in the Evelyn colorimeter with filter 440m μ . As checks, equivalent amounts of the synthetic triple salt solution were also diluted to 100 cc. and read at the same time.

acetic acid. The precipitate was separated by pouring the
tube first between the thumb and index finger of the left
hand and tapping the base with the right forefinger, thus
producing a rotary motion of the liquid in the tube. The
walls of the tubes were then washed down with about 1 cc. more
of liquid. The tubes were again centrifuged for 10 minutes,
decanted, inverted for five minutes, and dried as before.
The precipitate was then washed twice with 5 cc. portions of
ether to remove the acetic acid, which interferes with the
color with sulfanilic acid. After ether washing the
tubes were centrifuged for only 5 minutes and dried for
only one minute. Ether draining after ether washings may
allow the precipitate to become so dry that it falls out of
the tube. After draining from the second ether washing, the
tubes were placed over a steam radiator for 5 minutes to dry.
The contents of each tube were dissolved by centrifuging with
2-4 cc. of water, transferred quantitatively to a 100 cc.
volumetric flask, and diluted to about 70 cc. They were
added 4 cc. of 3% sulfanilic acid solution and 4 cc. of
10% acetic acid solution, and the solutions diluted to volume
and mixed. A 15 cc. portion of the solution in each flask
was transferred to an Erlenmeyer flask previously rinsed with the
solution, and the tubes were then in the water bath for
with 100 cc. of water. The solutions were also diluted to 100 cc.
and read at the same time.

Table 9

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride.

Sodium taken mgm.	Corrected galvanometer reading	Density	Sodium found mgm.	% error	K ob- ser- ved (from L&C)	K calc. (chart 5)	% Error
0	99 ²	.0022	.0024				
0	99 ³	.0011	.0012				
*.166	71 ²	.1457			.867	.871	-.5
*.166	71 ²	.1457			.867	.871	-.5
.168	71 ²	.1457	.166	-1.2			
.168	71 ¹	.1472	.168	0			
.173	70 ¹	.1534	.175	+1.2			
*.231	62 ³	.2024			.866	.860	+.7
*.231	62 ³	.2024			.866	.860	+.7
.231	62 ³	.2024	.231	0			
.231	63 ⁰	.2007	.229	-.8			
.238	62 ⁰	.2076	.237	-.4			
.238	62 ⁰	.2076	.237	-.4			
*.301	55 ¹	.2577			.856	.851	+.5
*.301	55 ¹	.2577			.856	.851	+.5
.301	55 ¹	.2577	.301	0			
.301	55 ¹	.2577	.301	0			
.301	55 ²	.2557	.299	-.8			
*.332	52 ¹	.2819			.848	.848	0
*.332	52 ¹	.2819			.848	.848	0

Table 9

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride.

Sodium taken mgm.	Corrected salinometer reading	Density	Sodium found mgm.	K error op- er- (al- v- (from 1%)	K error op- er- (al- v- (from 1%)	K error op- er- (al- v- (from 1%)
0	99	.0082	.0084			
0	99	.0081	.0082			
+.168	97	.1487				.887 .871 -.5
+.168	97	.1487				.887 .871 -.5
.168	97	.1487	.168	-1.2		
.168	97	.1472	.168	0		
.178	90	.1634	.178	+1.2		
+.231	89	.2024				.888 .880 +.7
+.231	89	.2024				.888 .880 +.7
.231	89	.2024	.231	0		
.231	89	.2007	.231	-.8		
.238	89	.2076	.237	-.4		
.238	89	.2076	.237	-.4		
+.301	85	.2877				.888 .881 +.5
+.301	85	.2877				.888 .881 +.5
.301	85	.2877	.301	0		
.301	85	.2877	.301	0		
.301	85	.2867	.298	-.8		
+.332	82	.2819				.848 .848 0
+.332	82	.2819				.848 .848 0

Table 9 (Cont.)

The efficiency of precipitation and washing.
Determination of known amounts of sodium chloride

Sodium taken mgm.	Corrected galvanometer reading	Density	Sodium found mgm.	% Error	K ob- ser- ved (from L&C)	K calc. (chart 5)	% Error
.332	52 ⁰	.2840	.334	+ .8			
.332	52 ¹	.2819	.332	0			
*.336	51 ³	.2861			.852	.847	+ .6
*.336	51 ³	.2861			.852	.847	+ .6
.336	52 ⁰	.2840	.334	- .7			
.336	51 ³	.2861	.336	0			
.336	52 ⁰	.2840	.334	- .7			
*.399	46 ¹	.335	.399		.839	.840	- .1
*.399	46 ¹	.335	.399		.839	.840	- .1
.399	46 ¹	.335	.399				
.399	46 ⁰	.337	.401	+ .6			
.399	46 ¹	.335	.399	0			
*.463	41 ⁰	.387			.838	.833	+ .6
.463	41 ⁰	.387	.463	0			
.463	41 ⁰	.387	.463	0			
.463	41 ⁰	.387	.463	0			
*.534	36 ⁰	.444			.832	.825	+ .8
*.534	36 ⁰	.444			.832	.825	+ .8
.534	36 ⁰	.444	.534	0			
.534	36 ⁰	.444	.534	0			

* Not precipitated or washed. Prepared from standard uranyl zinc sodium acetate solution.

Table 9 shows the results of these tests. It can be seen that the error in precipitation and washing averaged less than 1% within the ranges of .168 mgm. to .534 mgm. The last column of table 9 serves as a further check on the reproducibility of color of the triple salt with sulfosalicylic acid. No attempt was made in these experiments to study the precipitation and the washing separately, since the color produced by the precipitate can not be measured unless the excess of uranium is removed. These experiments show only that the errors in precipitation are offset by the errors in washing. However, the smallness of the error over the wide range would indicate that the error in either process is negligible .

Barber and Kolthoff (7) found that potassium, phosphate, and arsenates interfere in the gravimetric determination of sodium as uranyl zinc sodium acetate. In biological material the interference of arsenate may be ignored. Since potassium interferes only when it is present to the extent of 50 mgms. per cc. (7), it may be neglected in most biological fluids; however, since the concentration of potassium is much higher in cellular substance than in the surrounding fluids, it has been found advisable (67) to remove it by precipitation as perchlorate when cellular materials are analyzed. Since the present study is not concerned with such substances, the presence of potassium was ignored.

In the analysis of blood serum, the small concentration of phosphate can be ignored. In urine, however,

Table 2 shows the results of these tests. It can be seen that the error in precipitation and washing averaged less than 1% within the range of 1.58 mm to 1.04 mm. The last column of Table 2 serves as a further check on the reproducibility of color of the trials with self-indicating acid. No attempt was made in these experiments to study the precipitation and the washing separately, since the error involved by the precipitation can not be neglected unless the process of washing is removed. These experiments show only that the error in precipitation is offset by the error in washing. However, the analysis of the error over the wide range would indicate that the error in either process is negligible.

Berbat and Korbelt (7) found that potassium, phosphate, and arsenate interfere in the gravimetric determination of sodium as double and sodium acetate. In biological material the interference of arsenate may be ignored. Since potassium interferes only when it is present to the extent of 50 mg per cc. (7), it may be neglected in most biological fluids; however, since the concentration of potassium is much higher in cellular solutions than in the surrounding fluids, it has been found advisable (8) to remove it by precipitation as carbonate with cellular materials are analyzed. Since the present study is not concerned with such substances, the presence of potassium was ignored.

In the analysis of blood serum, the small concentration of phosphate can be ignored. In urine, however,

there is enough phosphate present to cause an appreciable precipitate. Butler and Tuthill (12), in applying the gravimetric method of Barber and Kolthoff (6), used solid calcium hydroxide for precipitating phosphate in preference to magnesia mixture, as recommended by the original workers (7). Overman and Garrett (69) used zinc carbonate. Hoffmann and Osgood (36), in developing a colorimetric method for sodium in urine, precipitated the phosphate along with the sodium, carried the insoluble uranyl phosphate through the washing process, and finally separated it by centrifuging after dissolving the sodium precipitate in water. Since these workers measured the color of the triple salt itself, which exhibits optimal color intensity when the precipitate from 0.1 cc. of urine is dissolved in 10 cc. or so of water, their procedure was very simple, consisting only of dissolving the precipitate in a measured volume of water, centrifuging down the insoluble uranyl phosphate, and reading the color of the supernatant fluid.

A modification of this method was used in the analyses of phosphate - containing solutions in the present study. Since the color developed from the triple salt with sulfo-salicylic acid required dilution to 100 cc. to obtain accuracy of reading, it was necessary to measure an aliquot part of the phosphate-free supernatant solution for color development and measurement. The validity of this procedure was tested in the experiment described below and

There is another phosphate present to cause an appreciable precipitate. Butler and Tschiff (12), in applying the gravimetric method of Garber and Kohnert (8), used solid calcium hydroxide for precipitating phosphate in preference to ammonia mixture, as recommended by the original workers (7). Overman and Garret (9) used zinc carbonate. Hoffmann and Orskov (36), in developing a colorimetric method for sodium in urine, precipitated the phosphate along with the sodium, carried the insoluble uranyl phosphate through the washing process, and finally separated it by centrifuging after dissolving the sodium phosphate in water. Since these workers measured the color of the triple salt itself, which exhibits optimal color intensity when the precipitate from 0.1 cc. of urine is dissolved in 10 cc. or so of water, their procedure was very simple, consisting only of dissolving the precipitate in a measured volume of water, centrifuging down the insoluble uranyl phosphate, and reading the color of the supernatant fluid. A modification of this method was used in the analyses of phosphate - containing solutions in the present study. Since the color developed from the triple salt with uranyl acetate is not retained in dilution to 100 cc. to obtain satisfactory readings, it was necessary to measure an aliquot part of the phosphate-free supernatant solution for color development and measurement. The validity of this procedure was tested in the experiment described below and

summarized in table 10.

A solution containing 1.0000 gm. of pure anhydrous Na_2HPO_4 per liter was prepared. This solution corresponded to a 1:10 dilution of urine containing twice the average concentration of phosphate; 1 L. contained .4999gm. P_{25} and 1 cc. contained .3239 mgm. of sodium. From one portion of this solution the phosphate was removed by the method of Butler and Tuthill (12); 18 cc. of solution was shaken with .06 gms. solid $\text{Ca}(\text{OH})_2$ for $\frac{1}{2}$ hour and filtered. A blank was prepared by shaking 18 cc. of water with .06 gms. $\text{Ca}(\text{OH})_2$. The solutions were filtered and 1 cc. portions were delivered into centrifuge tubes from a calibrated microburette. Samples of distilled water and of untreated sodium phosphate solution were also analyzed by the same procedure. The precipitation and washing were carried out in the same manner as with the sodium chloride solutions, except that in the washing the precipitates were agitated by stirring with a glass rod. This was found to be necessary because the bulky phosphate precipitate tended to pack down and occlude some of the reagent, as evidenced by high results. After the final ether washing, the precipitates were dried for five minutes and shaken vigorously with 10 cc. of water to dissolve the triple salt. After centrifuging for 10 minutes, 5 cc. of the clear supernatant fluids were transferred to 50 cc. volumetric flasks. The flasks were filled about two thirds full with water, 2 cc.

summarized in Table 10.

A solution containing 1.0000 gm. of pure anhydrous Na_2HPO_4 per liter was prepared. This solution corresponded to a 1:10 dilution of urine containing twice the average concentration of phosphate; 1 L. contained .4253 gm. P_2O_5 and 1 cc. contained .3839 mm. of sodium. From one portion of this solution the phosphate was removed by the method of Butler and Tschili (12); 18 cc. of solution was shaken with .05 gms. solid Ca(OH)_2 for $\frac{1}{2}$ hour and filtered. A blank was prepared by shaking 18 cc. of water with .05 gms. Ca(OH)_2 . The solutions were filtered and 1 cc. portions were delivered into centrifuge tubes from a calibrated microburette. Samples of distilled water and of untreated sodium phosphate solution were also analyzed by the same procedure. The precipitates and washings were carried out in the same manner as with the sodium chloride solutions, except that in the washing the precipitates were agitated by stirring with a glass rod. This was found to be necessary because the pink phosphate precipitates tended to pack down and occlude some of the reagent, as evidenced by blank results. After the final ether washing, the precipitates were dried for five minutes and shaken vigorously with 10 cc. of water to dissolve the triple salt. After centrifuging for 10 minutes, 1 cc. of the clear supernatant fluids were transferred to 50 cc. volumetric flasks. The flasks were filled about two thirds full with water, 2 cc.

Table 10

The non-interference of phosphates. Determination of sodium in disodium phosphate.

Sodium taken mgm.	Phosphate removed	Corrected galvanometer reading.	Density	Concentration of sodium C = L/K	Sodium found, mgm C-Blank	% Error
0	yes	99 ⁹	.0033	.004		
0	yes	99 ¹	.0033	.004		
.326	yes	52 ²	.2798	.330	.326	0
.326	yes	52 ²	.2798	.330	.326	0
.326	yes	52 ³	.2777	.328	.324	-.7
.326	yes	52 ¹	.2819	.332	.328	+.7
0	no	99 ¹	.0033	.004		
0	no	99 ¹	.0033	.004		
.326	no	52 ²	.2798	.330	.326	0
.326	no	52 ¹	.2819	.332	.328	+.7
.326	no	52 ³	.2777	.328	.324	-.7
.326	no	52 ²	.2798	.330	.326	0

Table 10

The non-influence of phosphate.
of sodium in aluminum phosphate.

Sodium taken, mm.	Phosphate removed	Corrected galvanometer reading.	Density	Concentration of sodium, mm.	Concentration of sodium, mm.	Determination
0	yes	99.9	.0035	.004		
0	yes	99.9	.0035	.004		
.325	yes	99.9	.2793	.330	.328	0
.325	yes	99.9	.2793	.330	.330	0
.325	yes	99.9	.2777	.328	.324	- .7
.325	yes	99.9	.2919	.325	.325	+ .7
0	no	99.9	.0035	.004		
0	no	99.9	.0035	.004		
.325	no	99.9	.2793	.330	.328	0
.325	no	99.9	.2819	.325	.328	+ .7
.325	no	99.9	.2777	.328	.324	- .7
.325	no	99.9	.2793	.330	.330	0

of sulfosalicylic acid and 2 cc. of sodium acetate were added to each, and 10-15 cc. of the solution read in the colorimeter as before. As shown in table 10, there was no difference in reliability between results obtained with and without removal of phosphates by precipitation with calcium hydroxide.

Since slightly acidified uranyl salts have been shown (34) to be excellent protein precipitants, it is necessary to remove or to destroy the protein in biological fluids before proceeding with the determination of sodium. The removal of protein by precipitation is far less time-consuming than is the destruction of protein by ashing, but the results of mineral determinations on deproteinized samples are not as reliable as those on ashed samples. The determinations of minerals in trichloroacetic acid filtrates have been studied extensively by Van Slyke, Hiller, and Berthelsen (95), Hald (35), Oberst (67), Grigant and Boutroux (34), and Ball and Sadusk (4). All these authors found that values obtained from trichloroacetic acid filtrates were from 2 to 5% higher than those from ashed samples, and all explained the error as due to volume changes caused by precipitating the protein. Some confirmation of this view is found in the results of Oberst (67), who found discrepancies of more than 5% between ashed and precipitated samples of cellular material, in which the protein content is higher than in plasma.

In urines, which contain little or no protein, precipitation by mercuric chloride (12) or by trichloroacetic acid (82)

of sodium acetate were added to each, and 10-15 cc. of the solution read in the colorimeter as before. As shown in table 10, there was no difference in reliability between results obtained with and without removal of phosphates by precipitation with calcium hydroxide.

Since slightly acidified urinary salts have been shown (34) to be excellent protein precipitants, it is necessary to remove or to destroy the protein in biological fluids before proceeding with the determination of sodium. The removal of protein by precipitation is far less time-consuming than is the destruction of protein by heating, but the results of

direct determinations on deproteinized samples are not as reliable as those on heated samples. The determination of chloride in uric acid samples has been studied extensively by Van Slyke, Miller, and Bartleson (35), and (36), Oser (37), and (38) and (39). All these authors found that values obtained from uric acid samples with filters were from 2 to 5% higher than those from heated samples, and all explained the error as due to volume changes caused by precipitation of the protein.

Some confirmation of this view is found in the results of Oser (37), who found discrepancies of more than 5% between heated and precipitated samples of certain material, in which the protein content is higher than in plasma.

In urines, which contain little or no protein, precipitation by uric acid (35) or by uric acid (36)

has not been found to introduce serious error. In the present study, 1:10 trichloroacetic acid filtrates of urine were made routinely. Details of the procedure and results will be shown in the next section.

When dealing with blood serum in the present study, determinations were made both on samples ashed by the procedure of Hoffman and Osgood (36) and on 1:10 trichloroacetic acid filtrates. Details of procedure and results are shown in the section dealing with the adaptation of the method to blood serum. Table 11 shows that values with trichloroacetic acid filtrates were from 0.9 to 4.0% higher than those on ashed samples of the same blood.

Application of method to biological fluids.

The theoretical, historical, and experimental basis for all the steps involved in the application of the method to blood, urine, and cerebrospinal fluid have been discussed in the preceding sections. The assembled procedures are presented here.

Reagents, used for sodium determinations in trichloroacetic acid filtrates of biological materials, are:

1. Trichloroacetic acid, 20% solution.
2. Ashless filter paper, Whatman No. 40.
3. Uranyl zinc acetate reagent, prepared according to Weinbach (96). Solution A: 77 gm. of uranyl acetate $\text{UO}_2 \cdot (\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, and 14 cc. of glacial acetic acid are dissolved by heating and stirring in 400 cc. of water. Solution B: 231 gm. of zinc acetate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, and 7 cc. of

has not been found to introduce serious error. In the present study, 1:10 trichloroacetic acid filtrates of uric acid were made. Details of the procedure and results will be shown in the next section.

When dealing with blood serum in the present study, determinations were made both on samples treated by the procedure of Hoffman and Nagao (24) and on 1:10 trichloroacetic acid filtrates. Details of procedure and results are shown in the next section dealing with the adaptation of the method to blood serum. Table II shows that values with trichloroacetic acid filtrates were from 0.7 to 0.9% higher than those on treated samples of the same blood.

Application of method to clinical fluids

The rheumatoid, hipical, and experimental data for all the steps involved in the application of the method to blood, urine, and cerebrospinal fluid have been discussed in the preceding sections. The essential procedures are presented here.

Reagents used for sodium hydroxide in solution:

acidic acid filtrates of biological materials are:

1. Trichloroacetic acid, 20% solution.

2. Acetic acid, 10% solution.

3. Uranyl zinc acetate reagent, prepared according

to Weinman (25), solution at 7% w/v of uranyl acetate in 10% acetic acid.

(CH₃COO)₂UO₂ and 1% w/v of alcohol soluble acid and dis-

solved by heating and filtering in 400 cc. of water. Solution

2:250 w/v of uric acid, 20(CH₃COO)₂UO₂ and 7 cc. of

glacial acetic acid are dissolved by heating and stirring in 400 cc. of water. The two solutions are mixed and diluted to 1 liter while still warm. After standing 24 hours, the mixed solution is ready for use. It is filtered immediately before using.

4. Ethyl alcohol, 95%.

5. Ethyl acetate-acetic acid wash reagent: 300 cc. of C. P. ethyl acetate diluted to 1 liter with glacial acetic acid.

6. Ethyl ether, redistilled.

7. Sulfosalicylic acid, 5% solution.

8. Sodium acetate, 10% solution: 50 gm. of C. P. sodium acetate, $\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$ dissolved in water and diluted to 500 cc.

Other reagents, used only in sodium determinations in ashed samples, are:

1. Sulfuric acid, 6N solution.

2. Nitric acid, concentrated.

3. Hydrogen peroxide, 30%.

For the determination of sodium in blood serum, urine or cerebrospinal fluid, 1 cc. of 1:10 trichloroacetic acid filtrate of the material or 0.1 cc. of material wet-washed by the procedure of Hoffman and Osgood (36) are analyzed.

In the preparation of trichloroacetic acid filtrates, 1 cc. of biological material is delivered from a pipette (calibrated

glacial acetic acid was dissolved by heating and stirring in 400 cc. of water. The two solutions are mixed and diluted to 1 liter while still warm. After standing 24 hours, the mixed solution is ready for use. It is filtered immediately before using.

4. Ethyl alcohol, 95%.

5. Ethyl acetate-acetic acid wash reagent: 300 cc. of C. P. ethyl acetate diluted to 1 liter with glacial acetic acid.

6. Ethyl ether, redistilled.

7. Sulfosalicylic acid, 5% solution.

8. Sodium acetate, 10% solution: 50 gm. of C. P. sodium acetate, NaCH_3COO , 5% dissolved in water and diluted to 500 cc.

Many reagents, used only in sodium determinations in

ashed samples, are:

1. Sulfuric acid, 6N solution.

2. Nitric acid, concentrated.

3. Hydrogen peroxide, 30%.

For the determination of sodium in blood serum, urine or cerebrospinal fluid, 1 cc. of 1:10 trichloroacetic acid 111-trace of the material or 0.1 cc. of acetone is wet-washed by the procedure of Hoffman and Osgood (36), and analyzed.

In the preparation of trichloroacetic acid 111-traces of biological material is delivered from a pipette (calibrated

to deliver the material measured) into 7 cc. of distilled water in a test tube. While shaking the tube gently, 2 cc. of 20% trichloroacetic acid is added. The contents of the tube are thoroughly mixed by stirring and allowed to stand for ten minutes. After filtering through an ashless filter paper, 1.0 cc. of the clear filtrate is delivered from a pipette calibrated for delivery of water into a 15 cc. conical centrifuge tube for treatment with uranyl zinc acetate reagent.

In the preparation of ashed samples, 0.1 cc. of the material to be analyzed is delivered from a pipette calibrated to deliver that material into a 15 cc. conical centrifuge tube of heat-resistant glass. After adding .2 cc. of 6N sulfuric acid and .1 cc. of concentrated nitric acid, the tube is placed in a beaker of boiling water for 10 minutes or longer. The tube is then removed from the water bath and the contents charred over a free flame. By allowing a small flame from the microburner to strike just above the level of the liquid in the constantly shaken tube, very little danger of loss from spattering is encountered. The tube is allowed to cool while another is being similarly treated. Then is added one drop of 30% H_2O_2 from a capillary pipette. The contents are again evaporated over a free flame until SO_3 fumes fill the tube, and allowed to cool during the treatment of the second tube. The addition of hydrogen peroxide is continued until, after evaporation to SO_3 fumes, only a colorless drop of liquid remains in the tube. (All tubes in any series are treated with equal

to deliver the material measured) into V cc. of distilled water in a test tube. While shaking the tube rapidly, 8 cc. of 30% trichloroacetic acid is added. The contents of the tube are thoroughly mixed by stirring and allowed to stand for ten minutes. After filtering through an ashless filter paper, 1.0 cc. of the clear filtrate is delivered from a hypodermic syringe into a 15 cc. conical centrifuge tube for treatment with uranyl zinc acetate reagent.

In the preparation of mixed samples, 0.1 cc. of the material to be analyzed is delivered from a pipette calibrated to deliver that material into a 15 cc. conical centrifuge tube of heat-resistant glass. After adding 2 cc. of 65 sulfuric acid and 1 cc. of concentrated nitric acid, the tube is placed in a beaker of boiling water for 10 minutes or longer. The tube is then removed from the water bath and the contents evaporated over a free flame. By allowing a small flame from the alcohol burner to strike just above the level of the liquid in the constantly shaken tube, very little danger of loss from spattering is encountered. The tube is allowed to cool while another is being similarly treated. Then is added one drop of 30% H₂O₂ from a capillary pipette. The contents are again evaporated over a free flame until 30% fumes fill the tube, and allowed to cool during the treatment of the second tube. The addition of hydrogen peroxide is continued until, after evaporation to 30% fumes, only a colorless drop of liquid remains in the tube. (All tubes in any series are treated with equal

amounts of H_2O_2 in order to insure constancy of the blank; 6 drops or less will generally be enough to produce a clear solution). After the final evaporation with H_2O_2 , the tube is allowed to cool for 10 minutes or more, and to it are added 0.9 cc. of water. After mixing the contents of the tube, it is ready for treatment with uranyl acetate reagent.

To 1 cc. of solution prepared by one of the methods described above, 5 cc. of freshly filtered uranyl zinc acetate reagent is added. At 5 minute intervals are added seven 0.13 cc. portions of ethyl alcohol. These additions must occupy at least $\frac{1}{2}$ hour, and may take longer. After each of the first five additions of alcohol the liquid in the tube is mixed, first by tapping the bottom of the tube, producing a rotatory motion in the upper part of the liquid, then by rolling the tube back and forth between the palms of the hands, thus producing effective mixing in the lowest part of the tube. The last two additions of alcohol serve to wash down the walls of the tube. They are allowed to remain layered on the solution. After the last addition of alcohol, the tube is centrifuged at 2000 r.p.m. for ten minutes, decanted, inverted, and allowed to drain for five minutes. The mouth of the tube is wiped dry and the precipitate is agitated by blowing on it a fine stream of about 2 cc. of ethyl acetate-acetic acid wash liquid*. The walls of

* In the case of urines which contain much phosphate the precipitate must be agitated by stirring with a glass rod, which is washed off with the wash liquid after each use.

amounts of H_2O in order to insure continuity of the blank; drops or less will generally be enough to produce a clear solution. After the final evaporation with H_2O , the tube is allowed to cool for 10 minutes or more, and to it are added 0.5 cc. of water. After mixing the contents of the tube, it is ready for treatment with ethyl acetate reagent.

To 1 cc. of solution prepared by one of the methods described above, 0.5 cc. of freshly filtered phenyl zinc acetate reagent is added. At 5 minute intervals are added seven 0.5 cc. portions of ethyl alcohol. These additions must occupy at least 1 hour, and may take longer. After each of the first five additions of alcohol the liquid in the tube is mixed, first by tapping the bottom of the tube, producing a rotary motion in the upper part of the liquid, then by rotating the tube back and forth between the palms of the hands, thus producing a rotary motion in the lower part of the tube. The last two additions of alcohol serve to wash down the walls of the tube. They are allowed to remain layered on the solution. After the last addition of alcohol, the tube is desiccated at 1000 mm. for ten minutes, exhausted, inverted, and allowed to drain for five minutes. The mouth of the tube is wiped dry and the precipitate is extracted by blowing on it a fine stream of cold 5 cc. of ethyl acetate-acetic acid wash liquid. The walls of

* In the case of uric acid which contains much phosphate the precipitate must be extracted by stirring with a glass rod, which is washed off with the wash liquid after each use.

the tube are washed down with a small amount of the liquid. Centrifuging for ten minutes, draining for five minutes, and wiping are carried out as before, and about 5 cc. of ether are used to wash the precipitate and the walls of the tube; precipitates from urines must be stirred with a glass rod. The tube is centrifuged for 5 minutes, decanted, and drained for 1 minute. (Longer draining may allow the flaky precipitate to become so dry that it crumbles and falls out of the tube). The precipitate is washed a second time with 5 cc. of ether, centrifuged 5 minutes, decanted, and drained for one minute. The tube is placed in a warm place for 5 minutes to evaporate the last traces of ether. To this point the procedure is identical for blood, urine, and cerebrospinal fluids, with the exception of the agitation of precipitates, as noted.

For blood and cerebrospinal fluid, the remainder of the procedure is identical: the washed, dried precipitate is dissolved in 4-5 cc. of water and transferred quantitatively to a 100 cc. volumetric flask. The solution in the flask is diluted to about 70 cc., and to it are added in order 4 cc. of 5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and water to make 100 cc. The contents of the flask are mixed thoroughly and a 15 cc. portion transferred to an Evelyn colorimeter tube and read in the colorimeter, using the 440 m μ filter. The instrument is set to read 100 with a tube containing 4 cc. of each of 5% sulfosalicylic acid and 10% sodium acetate in 100 cc. The L value corresponding to

The tube are washed down with a small amount of the liquid.
Centrifuging for 10 minutes, draining for five minutes, and
wiping are carried out as before, and about 5 cc. of ether
are used to wash the precipitate and the side of the tube;
precipitate from urines must be stirred with a glass rod.
The tube is centrifuged for 5 minutes, decanted, and drained
for 1 minute. (Larger draining may allow the fatty precipitate
to become so dry that it crumbles and falls out of the tube).
The precipitate is washed a second time with 5 cc. of ether,
centrifuged 5 minutes, decanted, and drained for one minute.
The tube is placed in a warm place for 5 minutes to evaporate
the last traces of ether. To this point the procedure is
identical for blood, urine, and cerebrospinal fluids, with the
exception of the addition of phosphates, as noted.
For blood and cerebrospinal fluids, the remainder of the
procedure is identical: the washed, dried precipitate is
dissolved in 4-5 cc. of water and transferred quantitatively
to a 100 cc. volumetric flask. The solution in the flask
is diluted to about 75 cc., and 10 cc. are added in either 5 cc.
of 5% sulfosalicylic acid, 4 cc. of 10% sodium acetate, and
water to make 100 cc. The contents of the flask are mixed
thoroughly and a 10 cc. portion transferred to an Evelyn
colorimeter tube and read in the colorimeter, using the 480 mμ
filter. The instrument is set to read 100 with a tube con-
taining 4 cc. of each of 5% sulfosalicylic acid and 10%
sodium acetate in 100 cc. The values corresponding to

the corrected galvanometer reading is read from the chart supplied with the colorimeter, and the concentration of sodium in the original material is calculated from the concentration in the sample tube (read from chart 7) by subtracting the sodium content of a similarly treated blank*.

If c is the concentration of sodium in the sample tube, b is the concentration of sodium in the blank tube (obtained by dividing L by the correct value of K from chart 5), and v is the volume of serum or spinal fluid taken in the ashed sample or trichloroacetic acid filtrate, then

$$\frac{100(c-b)}{v} = \text{mgms. \% of sodium in serum or spinal fluid.}$$

The precipitate from urine is shaken with 10 cc. of water; the triple salt dissolves while the uranyl phosphate remains behind as a gelatinous precipitate. The tube is centrifuged for ten minutes at 200 r.p.m. Five cubic centimeters of the clear supernatant fluid is pipetted into a 50 cc. volumetric flask and diluted to about 35 cc. with water. Then are added in order 2 cc. of 5% sulfosalicylic acid, 2 cc. of 10% sodium acetate, and water to make 50 cc. The solution is read in the colorimeter in the same manner as is a solution of blood precipitate. Since the colored solution contained

* The reagent blank for trichloroacetic acid filtrates is prepared by diluting two parts of 20% trichloroacetic acid with 8 parts of water, filtering, adding 1 cc. to a centrifuged tube, and carrying out the precipitation and washing as with blood filtrates. The blank for ashed samples is prepared by treating 0.1 cc. of water with sulfuric acid, nitric acid, and hydrogen peroxide, and carrying out the procedure exactly as with blood serum.

the corrected galvanometer reading is read from the chart supplied with the colorimeter, and the concentration of sodium in the original material is calculated from the concentration in the sample tube (read from chart 7) by subtracting the sodium content of a similarly treated blank.

If c is the concentration of sodium in the sample tube, b is the concentration of sodium in the blank tube (obtained by dividing b by the correct value of k from chart 5), and v is the volume of serum or spinal fluid taken in the shaded sample or blank colorimetric cell, then

$$\frac{100(c-b)}{v} = \text{mM. \% of sodium in serum or spinal fluid.}$$

The precipitate from urine is shaken with 10 cc. of water; the triple salt dissolves while the uric acid precipitate remains behind as a refractory precipitate. The case is centrifuged for ten minutes at 800 r.p.m. Five cubic centimeters of the clear supernatant fluid is pipetted into a 50 cc. volumetric flask and diluted to about 50 cc. with water. Then are added in order 2 cc. of 2% antichlorogenic acid, 2 cc. of 10% sodium acetate, and water to make 50 cc. The solution is read in the colorimeter in the same manner as is a solution of blood precipitate. Since the colored solution contained

The reagent blank for antichlorogenic acid and uric acid is prepared by diluting two parts of 10% antichlorogenic acid with 3 parts of water, filtering, adding 1 cc. of a concentrated cube, and carrying out the precipitation and washing as with blood filtrate. The blank for added uric acid is prepared by treating 0.1 cc. of water with antichlorogenic acid, uric acid, and hydrogen peroxide, and carrying out the procedure exactly as with blood serum.

half the precipitate from the sample in 50 cc. of final volume, the concentration of color is the same as if all the precipitate was dissolved in 100cc., so the same charts can be used for finding the concentrations of sodium in sample and blank, and the calculation of results is the same as for blood or cerebrospinal fluid.

Table 11 shows the results of analyses of fifteen samples of blood. Ashed samples and trichloroacetic acid filtrates of each blood were prepared and were analyzed by the procedure given above. The average value of the sodium concentration in 15 normal blood sera, determined on ashed samples, was found to be 334 mgms. per 100 cc. The range was from 324 to 342 mgms. per 100 cc. As determined on trichloroacetic acid filtrates, the average was 341 mgms. per 100cc. and the range from 333 to 349 mgms. per 100 cc. The average value for ashed samples is in excellent agreement with the figure of .335 mgms. per 100cc., which Peters and Van Slyke (71) give as the normal concentration. The variation of the extremes was less than $\pm 4\%$, which Hoffman and Osgood (36) state as the normal variation of human blood sodium.

Trichloroacetic acid filtrates gave values from .9 to 4.0% higher than ashed samples of the same blood. The average difference was 2.3%. This figure compares favorably with those reported by Van Slyke, Hiller and Berthelsen (95), Hald (35), Grigant and Boutroux (34), and Ball and Sadusk (4).

Table 12 shows the results of the analyses of ten samples

half the precipitate from the sample in 50 cc. of fluid volume
the concentration of color is the same as if all the precipi-
tate was dissolved in 100 cc., so the same device can be used
for finding the concentrations of sodium in sample and blank,
and the calculation of results is the same as for blood or
serum samples.

Table II shows the results of analyses of fifteen samples
of blood. Assay samples and indicator were added in 100 cc. of
each blood were prepared and were analyzed by the procedure
given above. The average value of the sodium concentration
in 15 normal blood sera, determined on serum samples, was
found to be 325 mEq. per 100 cc. The range was from 324 to
325 mEq. per 100 cc. as determined on triplicate sera
samples, the average was 325 mEq. per 100 cc. and the range
from 324 to 325 mEq. per 100 cc. The average value for assayed
samples is in excellent agreement with the value of 325
mEq. per 100 cc., which value and Van Slyke (2) give as
the normal concentration. The variation of the experiment was
less than 1%, which is better than the 2% which is the
normal variation of human triplicate samples.

Triphosphate salts in blood have values from 1.5 to 4.0%
higher than values found in the same blood. The average dif-
ference was 2.5%. This value agrees favorably with those
reported by Van Slyke, Miller and Harrison (3), 2.5% (33),
Sylvester and Harrison (4), and Hall and Sargent (5).

Table II shows the results of the analyses of ten samples

Table 11

Determination of sodium in blood sera.

No.	Blood taken cc.	Treatment *	L**	Total sodium found mgms.	Sodium in blood, mgms. (total - blank)	Sodium in blood mgms. %	% diff. A & T
0	0	A	.0088	.020			
	0	T	.0066	.014			
1	.200	A	.2924	.693	.673	337.	
	.202	T	.297	.702	.688	341.	1.2
	.202	T	.297	.702	.688	341.	1.2
2	.200	A	.2882	.682	.662	331.	
	.200	A	.2882	.682	.662	331.	
	.202	T	.2903	.688	.674	334.	.9
	.202	T	.2903	.688	.674	334.	.9
3	.200	A	.2882	.682	.662	331.	
	.202	T	.297	.704	.690	342.	3.3
	.202	T	.297	.704	.690	342.	3.3
4	.200	A	.2903	.688	.668	334.	
	.200	A	.2882	.682	.662	331.	
	.202	T	.299	.710	.696	345.	3.8
	.202	T	.297	.704	.690	342.	2.8
5	.200	A	.2924	.693	.673	337.	
	.202	T	.297	.704	.690	342.	1.5
	.202	T	.297	.704	.690	342.	1.5
6	.200	A	.297	.704	.684	342.	
	.200	A	.297	.704	.684	342.	
	.202	T	.305	.720	.706	349.	2.0
	.202	T	.305	.720	.706	349.	2.0
7	.200	A	.295	.700	.680	340.	
	.202	T	.299	.710	.696	345.	1.5
8	.200	A	.2924	.693	.673	337.	
	.200	A	.2924	.693	.673	337.	
	.202	T	.299	.710	.696	345.	2.4
	.202	T	.299	.710	.696	345.	2.4

* A = ashed. T = trichloroacetic acid.

** Final volume of colored solution was 200 cc.

Table 11 (cont.)

Determination of sodium in blood sera.

No.	Blood taken cc.	Treat- ment *	L**	Total sodium found, mgms.	Sodium in blood, (total- blank)	Sodium in blood, mgms. %	% diff. A & T
00	0	A	.0044	.005			
	0	T	.0144	.016			
9	.103	A	.2882	.341	.336	326.	
	.103	A	.2861	.339	.334	324.	
	.101	T	.299	.355	.339	336.	3.4
	.101	T	.301	.357	.341	338.	4.0
10	.103	A	.2882	.341	.336	326.	
	.103	A	.2882	.341	.336	326.	
	.101	T	.301	.357	.341	338.	3.7
	.101	T	.301	.357	.341	338.	3.7
11	.103	A	.2924	.347	.342	332.	
	.103	A	.2924	.347	.342	332.	
	.101	T	.305	.363	.347	344.	3.6
	.101	T	.305	.363	.347	344.	3.6
12	.103	A	.295	.350	.345	335.	
	.103	A	.295	.350	.345	335.	
	.101	T	.305	.363	.347	344.	2.8
13	.103	A	.2903	.344	.339	329.	
	.103	A	.2882	.341	.336	326.	
	.101	T	.297	.352	.336	333.	1.7
	.101	T	.297	.352	.336	333.	1.7
000	0	A	.0166	.019			
	0	T	.0121	.014			
14	.097	A	.2903	.344	.325	335.	
	.101	T	.299	.355	.341	338.	.9
15	.097	A	.2903	.344	.325	335.	
	.097	A	.2903	.344	.325	335.	
	.101	T	.301	.358	.344	341.	1.8
	.101	T	.299	.355	.341	338.	.9

* A ashed. T = trichloroacetic acid.

** Final volume of colored solution was 100cc.

Table 12

Determination of sodium in urines						
No.	Urine taken, cc.	Treatment *	L	Total sodium found, mgms.	Sodium in urine, mgms.	Sodium in urine, mgms. %
0	0	A	.0044	.005		
	0	T	.0144	.016		
1	.103	A	.328	.389	.384	374
	.103	A	.332	.394	.389	378
	.101	T	.342	.407	.391	387
	.101	T	.342	.407	.391	387
2	.103	A	.409	.493	.488	475
	.103	A	.406	.489	.484	471
	.101	T	.426	.514	.498	493
	.101	T	.423	.511	.495	490
3	.101	T	.349	.416	.400	396
4	.101	T	.1565	.180	.164	162
	.101	T	.1565	.180	.164	162
5	.101	T	.1192	.137	.121	120
	.101	T	.1192	.137	.121	120
6	.101	T	.1805	.208	.192	190
	.101	T	.1821	.210	.194	192
7	.101	T	.1905	.221	.205	203
	.101	T	.1888	.219	.203	201
8	.101	T	.2007	.233	.217	215
	.101	T	.1973	.230	.214	212
9	.101	T	.1739	.202	.188	186
	.101	T	.1739	.202	.188	186
10	.101	T	.1805	.208	.192	190
	.101	T	.1821	.210	.194	190

* A = ashed. T = trichloroacetic acid.

of urine. Excellent agreement was found between duplicate determinations on both trichloroacetic acid filtrates and ashed samples, but the determinations on trichloroacetic acid filtrates, performed on specimens 1 and 2 gave results about 4% higher than those on ashed samples. This discrepancy does not seem explainable on the basis of any protein present, as the precipitate obtained in both cases was negligible. The agreement of duplicates in the range of 120 to 493 mgms. % of sodium per 100 cc. indicates that the method is applicable to samples of urine with sodium concentrations in this range.

Two samples of cerebrospinal fluid were analyzed, both by ashed samples and trichloroacetic acid filtrates. The results, tabulated in table 13, likewise show higher values (average 2.3%) for trichloroacetic acid filtrates than for ashed samples. The actual values are comparable with those of blood sera.

The accuracy of the method as applied to biological fluids is demonstrated by the agreement between duplicate samples treated in the same way. As an absolute check on the accuracy, known amounts of sodium chloride were carried through the entire procedure, including ashing and preparing the filtrate. Sodium was recovered from ashed samples with errors of $-.3\%$ and $+.6\%$ from trichloroacetic acid filtrates with errors of 0.0 and $+.6\%$ as shown in table 14.

Four experiments were performed in which known amounts of sodium chloride and of analyzed blood were ashed and carried through the procedure. Sodium was recovered with errors of 0.0, $+.3$, and $+.3\%$. Results are shown in table 14.

of urine. Excellent agreement was found between duplicate determinations on both trichloroacetic acid filtrates and unacidified samples, but the determinations on trichloroacetic acid filtrates, performed on specimens 1 and 2, gave results about 4% higher than those on unacidified samples. This discrepancy does not seem explainable on the basis of any gross error, as the precipitate obtained in both cases was negligible. The agreement of duplicate in the range of 180 to 400 μ moles of sodium per 100 cc. indicates that the method is applicable to samples of urine with sodium concentrations in this range.

Two samples of cerebrospinal fluid were analyzed, both by unacidified and trichloroacetic acid filtrates. The results, tabulated in table 13, likewise show higher values (average 2.3%) for trichloroacetic acid filtrates than for unacidified samples. The actual values are comparable with those of blood sera.

The accuracy of the method as applied to biological fluids is demonstrated by the agreement between duplicate samples titrated in the same way. As an absolute check on the accuracy, known amounts of sodium chloride were added through the entire procedure, including sample and reagent filtrates. Sodium was recovered from unacid samples with errors of -0.3% and +0.6% from trichloroacetic acid filtrates with errors of 0.0% and +0.6% as shown in table 14.

Four experiments were performed in which known amounts of sodium chloride and of unacid blood sera were added and carried through the procedure. Sodium was recovered with errors of -0.0%, +0.3%, +0.4% and +0.5%. The results are shown in table 14.

Table 13

Determination of sodium in cerebrospinal fluids.

No.	C. S. F. taken, cc.	Treatment*	L	Total sodium found, mgms.	Sodium in C.S. F. mgms. (Total-blank)	Sodium in C. S. F. mgms. %
0	0	A	.0044	.005		
	0	A	.0144	.016		
1	.103	A	.295	.350	.345	335.
	.103	A	.295	.350	.345	335.
	.101	T	.303	.360	.344	341.
	.101	T	.303	.360	.344	341.
2	.103	A	.2924	.347	.342	332.
	.101	T	.303	.360	.344	341.
	.101	T	.301	.357	.341	338.

*A = ashed. T= trichloroacetic acid.

Table 13

Determination of sodium in ceropogonin fluids.

No.	G. S. F. taken, cc.	Treatment	Total sodium found, mms.	Sodium in G. S. F. mms. (Total-blank)	Sodium in G. S. F. mms.
0	0	A	.0044	.003	
	0	A	.0144	.018	
1	.103	A	.285	.350	.350.
	.103	A	.285	.350	.350.
	.101	T	.303	.360	.361.
	.101	T	.303	.360	.361.
2	.103	A	.285	.357	.355.
	.101	T	.303	.360	.361.
	.101	T	.301	.357	.358.

*A = ashed. T = trichloroacetic acid.

Table 14

Recovery of sodium from mixtures of analyzed
blood and standard sodium chloride solution

Treat- ment*	Sodium taken, mgm.		Volume L final soln., cc.	Total sodium found, mgm.	Sodium from NaCl= total- blood- blank	% error
	from NaCl	from blood				
A	0	0	100	.0044	.005	
A	.340	0	100	.2903	.344	.339
A	.340	0	100	.2924	.347	.342
						- .3
						+ .6
A	0	**	100	.2882	.341	.336) from
A	0	**	100	.2882	.341	.336) blood
A	.340	.336	200	.2882	.681	.340
A	.340	.336	200	.2882	.681	.340
						0
						0
A	0	**	100	.2924	.347	.342) from
A	0	**	100	.2924	.347	.342) blood
A	.340	.342	200	.2903	.688	.341
A	.340	.342	200	.2903	.688	.341
						+ .3
						+ .3
T	0	0	100	.0144	.016	
T	.334	0	100	.295	.350	.334
T	.334	0	100	.297	.352	.336
						0
						+ .6

* A = ashed. T = trichloroacetic acid.

** Same quantity (.103 cc.) of blood taken here as added in following two recovery experiments.

Table 1A

Recovery of sodium from mixtures of dehydrated blood and standard sodium chloride solution

Test- tube	Sodium from blood	Sodium from standard	Total sodium	Recovery percent	Standard error
1	0	100	100	100	0
2	0	100	100	100	0
3	0	100	100	100	0
4	0	100	100	100	0
5	0	100	100	100	0
6	0	100	100	100	0
7	0	100	100	100	0
8	0	100	100	100	0
9	0	100	100	100	0
10	0	100	100	100	0
11	0	100	100	100	0
12	0	100	100	100	0
13	0	100	100	100	0
14	0	100	100	100	0
15	0	100	100	100	0
16	0	100	100	100	0
17	0	100	100	100	0
18	0	100	100	100	0
19	0	100	100	100	0
20	0	100	100	100	0
21	0	100	100	100	0
22	0	100	100	100	0
23	0	100	100	100	0
24	0	100	100	100	0
25	0	100	100	100	0
26	0	100	100	100	0
27	0	100	100	100	0
28	0	100	100	100	0
29	0	100	100	100	0
30	0	100	100	100	0
31	0	100	100	100	0
32	0	100	100	100	0
33	0	100	100	100	0
34	0	100	100	100	0
35	0	100	100	100	0
36	0	100	100	100	0
37	0	100	100	100	0
38	0	100	100	100	0
39	0	100	100	100	0
40	0	100	100	100	0
41	0	100	100	100	0
42	0	100	100	100	0
43	0	100	100	100	0
44	0	100	100	100	0
45	0	100	100	100	0
46	0	100	100	100	0
47	0	100	100	100	0
48	0	100	100	100	0
49	0	100	100	100	0
50	0	100	100	100	0

10 = added, 12 = trichloroacetic acid.

Same quantity (.10% cc.) of blood taken here as added in following two quantity experiments.

Summary

1. The limitations of existing methods for the determination of sodium in biological fluids have been discussed.
2. It has been shown that the Evelyn colorimeter does not detect differences in concentration of less than .6%.
3. The color developed from the uranyl ion with sulfosalicylic acid and sodium acetate has been studied. The conformity of this color with Beer's law has been studied, and the color has been shown to be reproducible and to be stable with respect to temperature and time.
4. A new method, consisting of precipitation of uranyl zinc sodium acetate from alcoholic solution, removal of excess precipitant by washing with ethyl acetate in acetic acid and with ether, removal of phosphate as uranyl phosphate, and photoelectric measurement of the color developed with sulfosalicylic acid and sodium acetate, has been developed for the determination of sodium in biological fluids.
5. Recovery of sodium from known amounts of sodium chloride, both in simple aqueous solution and added to biological fluids, has indicated that the various steps, as well as the assembled procedure, have a maximum error of less than 1%.

1. The limitations of existing methods for the determination of sodium in biological fluids have been discussed.
2. It has been shown that the Evelyn colorimeter does not detect differences in concentration of less than 0.5%.
3. The color developed from the urinary ion with antipyrinic acid and sodium acetate has been studied. The conformity of this color with Beer's law has been studied, and the color has been shown to be reproducible and to be stable with respect to temperature and time.
4. A new method, consisting of precipitation of urinary zinc sodium acetate from alcoholic solution, removal of excess precipitant by washing with ethyl acetate in aqueous acid and with ether, removal of phosphate as methyl phosphate, and photometric measurement of the color developed with antipyrinic acid and sodium acetate, has been developed for the determination of sodium in biological fluids.
5. Recovery of sodium from known amounts of sodium chloride, both in simple aqueous solution and added to biological fluids, has indicated that the various steps, as well as the assembled procedure, have a relative error of less than 1%.

Abstract

A calculation of the percentage of error involved in the use of the Evelyn photoelectric colorimeter has shown that since the percentage of light transmitted by a colored solution can be measured to within one-quarter of one percent and since the light transmitted is a logarithmic function of the concentration of absorbing material, the error can be held to a maximum of .6 - .7% by the proper choice of sizes of samples. This accuracy, together with the saving of time effected by photoelectric colorimetry as compared with titrations or weighings, creates a definite demand for a photoelectric method for the determination of sodium. Since the normal variation in concentration of sodium in human blood serum ranges only four percent in either direction from the mean value, a method for the clinical determination of sodium must have an error not greater than one percent.

A review of the literature reveals only one photoelectric method for the determination of sodium. This method, published by Hoffman and Osgood, is based on the precipitation of sodium as uranyl zinc sodium acetate, the solution of the precipitate, and the photoelectric measurement of the yellow color of the uranyl ion. The authors found that the color is relatively unstable to heat and to acidity.

Preliminary experiments on the colors imparted by phenolic

acids to uranyl salts showed that sulfosalicylic acid, a common, colorless, very soluble reagent, imparts to uranyl salts an orange color exhibiting a narrow absorption band with maximum absorption at 455 millimicrons. Further investigation showed that the color can be intensified by the addition of sodium acetate. The color conforms with Beer's law to well within one percent over ranges of concentration of uranium of ten percent. Over a wide range of concentration the deviation from Beer's law is apparently directly proportional to the concentration of uranium, so that a calculation of concentration from color density is much simplified. The color is reproducible, stable over a period of three hours, and is not markedly affected by changes of temperature. The color is somewhat unstable to changes of acidity. Careful standardization of the color reagents eliminated any errors from this source.

All methods so far proposed for the washing of the precipitated uranyl zinc sodium acetate have called for the use of liquids saturated with the triple salt. Hoffman and Osgood showed that these wash reagents are objectionable because some of the triple salt is precipitated from them when they are brought in contact with uranyl zinc acetate. The use of the precipitation method of Weinbach and of a new wash reagent

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as follows: the sample is prepared for analysis by the re-
containing ethyl acetate in acetic acid eliminated this ex-
cessive precipitation and afforded quantitative recovery at
room temperature of sodium in amounts ranging from 0.165 to
0.534 milligrams.

Since uranyl salts precipitate phosphate as an extremely
insoluble uranyl phosphate, previous workers have removed phos-
phate from urines, where its concentration may be high, before
precipitating the sodium. The phosphate content of blood and
cerebrospinal fluid is insufficient to interfere with the de-
termination of sodium. In the analysis of urines in the
present study phosphate was precipitated along with the sodium
by the uranium, and was carried through the washing process
with the sodium compound. It was finally removed by dissolving
the triple salt in a measured quantity of water, centrifuging
off the insoluble uranyl phosphate, and taking an aliquot part
of the clear supernatant fluid for completion of the analysis.
This procedure was modified from that of Hoffman and Osgood.
Determinations of sodium in solutions of disodium phosphate
having a concentration of phosphate twice that encountered in
urine indicated that this procedure gives results as reliable
as those given by procedures in which phosphate is removed,
provided that care be taken, in washing the precipitate, to re-
move any occluded uranium.

The procedure for the determination of sodium is

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as follows: the sample is prepared for analysis by the removal of protein either by wet ashing or by treatment with trichloroacetic acid. In the wet ashing process of Hoffman and Osgood, used in these experiments, 0.1 cc. of the material is treated in a 15 cc. conical centrifuge tube of heat-resistant glass with .2 cc. of 6 N sulfuric acid and .1 cc. of concentrated nitric acid and, after evaporating the solution to SO_3 fumes before each addition, 6 drops of 30% H_2O_2 , added one drop at a time. After the final addition of H_2O_2 the solution is evaporated to SO_3 fumes, cooled, and diluted with .9 cc. of water. Trichloroacetic acid filtrates are prepared by mixing 1.0 cc. of biological material with 7.0 cc. of water and 2.0 cc. of 20% trichloroacetic acid. After standing for 10 minutes the solution is filtered through an ashless filter paper. One cubic centimeter of the filtrate is transferred to a 15 cc. centrifuge tube for analysis. According to the method used above, 0.1 cc. or 1.0 cc. of water is treated in exactly the same way for the reagent blank.

The precipitation is accomplished by adding to the 1 cc. sample (equivalent to 0.1 cc. of biological material) 5 cc. of Weinbach's uranyl zinc acetate reagent and seven 0.3 cc. portions of 95% ethyl alcohol. The additions of alcohol are spread over one-half hour or more, and the alcohol is thoroughly mixed with the liquid in the tube after the first five additions; the last two portions of alcohol serve to wash

as follows: the sample is prepared for analysis by the removal of protein either by wet ashing or by treatment with trichloroacetic acid. In the wet ashing process of Hoffman and Garrod, used in these experiments, 0.1 cc. of the material is treated in a 10 cc. conical centrifuge tube of heat-resistant glass with 2 cc. of 6 N sulfuric acid and 1 cc. of concentrated nitric acid and, after evaporating the solution to 30 times before each addition, a drop of 30% H_2O_2 is added one drop at a time. After the final addition of H_2O_2 the solution is evaporated to 30 times, cooled, and diluted with 5 cc. of water. Trichloroacetic acid filtrates are prepared by mixing 1.0 cc. of biological material with 5.0 cc. of water and 2.0 cc. of 20% trichloroacetic acid. After standing for 10 minutes the solution is filtered through an anhydrous filter paper. One cubic centimeter of the filtrate is transferred to a 10 cc. centrifuge tube for analysis. According to the method used above, 0.1 cc. of 1.0 cc. of water is pressed in exactly the same way for the reagent blank.

The precipitation is accomplished by adding to the 1 cc. sample (equivalent to 0.1 cc. of biological material) 5 cc. of Watson's uranyl zinc acetate reagent and seven 0.5 cc. portions of 95% ethyl alcohol. The additions of alcohol are spread over one-half hour or more, and the alcohol is thoroughly mixed with the liquid in the tube after the first five additions; the last two portions of alcohol serve to wash

down the walls of the tube. The tube is centrifuged for 10 minutes at 2000 r.p.m., the supernatant fluid decanted, and the tube inverted and allowed to drain for 5 minutes. The precipitate is washed by blowing onto it about 2 cc. of 30 % ethyl acetate in acetic acid. After agitating the precipitate in the wash liquid, the walls of the tube are washed down with a small portion of the liquid. The tube is again centrifuged for 10 minutes and drained for 5 minutes. Washing with 5 cc. of ether, centrifuging 5 minutes, and draining 1 minute are carried out twice. The tube is placed upright in a warm place for 5 minutes to remove the last traces of ether. If the phosphate content of the original material is negligible, the precipitate is dissolved in water, transferred quantitatively to a 100 cc. volumetric flask, diluted to about 70 cc. with water, mixed with 4 cc. each of 5 % sulfosalicylic acid and of 10 % sodium acetate, and diluted to the mark. Precipitates from phosphate-containing fluids are shaken with 10 cc. of water, which dissolves the triple salt. The insoluble uranyl phosphate is centrifuged off and 5 cc. of the supernatant fluid is transferred to a 50 cc. volumetric flask, treated with half quantities of the color reagents, and diluted to the mark. A color blank is prepared by diluting 4 cc. of each of the color reagents to 100 cc. with water. Portions of 15 cc. from each of the flasks — biological fluid, reagent blank, and color blank — are transferred to Evelyn colorimeter tubes and read in the Evelyn

down the walls of the tube. The tube is centrifuged for
10 minutes at 3000 r.p.m., the supernatant fluid removed,
and the tube inverted and allowed to drain for 5 minutes.
The precipitate is washed by blowing twice with 5 cc. of
50% ethanol acetate in acetic acid. After repeating the pro-
cedure in the same liquid, the walls of the tube are washed
down with a small portion of the liquid. The tube is again
centrifuged for 10 minutes and drained for 5 minutes. Washing
with 5 cc. of ether, centrifuging 5 minutes, and draining
1 minute are repeated one more time. The tube is placed upright
in a warm place for 5 minutes to remove the last traces of
ether. If the phosphate content of the original material is
negligible, the precipitate is dissolved in water, filtered,
dried quantitatively to a 100 cc. volumetric flask, diluted
to about 90 cc. with water, dried with 5 cc. of alcohol of 95%
ethanol, dried with one or two portions of 10% ethanol acetate, and diluted
to the mark. From this two phosphate-containing liquids
are obtained with 10 cc. of water, which dissolves the residue
left. The insoluble material is removed by centrifuging 5% and
5 cc. of the supernatant fluid is transferred to 10 cc.
volumetric flask, dried with 5 cc. of ethanol acetate, and diluted
to the mark, and added to the 10 cc. of water. The liquid is
of volume 10 cc. and used for the determination of 100 cc.
with water. The volume of 10 cc. from each of the liquids —
of identical fluid, one for blank, and other blank — are trans-
ferred to 100 cc. volumetric flasks and read in the Evelyn

photoelectric colorimeter, using a blue filter showing maximum transmission at 440 millimicrons. The galvanometer is set to read 100 with the color blank, and readings are taken on the other tubes. From the galvanometer readings the color densities of the tubes are calculated. The sodium concentration in each tube is calculated from the experimentally determined relationship of concentration to density. Where L is the "optical density" ($2 - \log G$) and C is the concentration of sodium in milligrams per 100 cc. of colored solution, $L/C = .848$ in the ranges encountered in the analyses of blood. By subtracting the concentration of sodium in the reagent blank from that in the sample tube and dividing by the volume of biological fluid taken for analysis, the amount of sodium in 1 cc. of fluid is obtained.

The sodium concentration in 15 normal human blood sera, determined on ashed samples, was found to vary between 324 and 342 (average 334) mgms. per 100cc. Values obtained on trichloroacetic acid filtrates averaged 2.3% higher. Concentrations observed on 2 samples of cerebrospinal fluid were comparable to those observed in blood sera. Samples of ten urines varied in sodium content between 162 and 493 milligrams per 100 cc.

Analyses of known amounts of sodium chloride, both in simple aqueous solution and mixed with measured amounts of analyzed blood serum, indicated that the error involved in the use of the method varies from $-.3\%$ to $+.6\%$.

photocoloric colorimeter, using a blue filter showing an absorption maximum at 440 millimicrons. The colorimeter is set to read 100 when the color blank, and readings are taken on the other tubes. From the colorimeter readings the color densities of the tubes are calculated. The sodium concentration in each tube is calculated from the experimentally determined relationship of concentration to density. These are the "optical density" (D-log D) and C is the concentration of sodium in milligrams per 100 cc. of colored solution, $D/C = 0.54$ in the range encountered in the analysis of blood. By subtracting the concentration of sodium in the reagent blank from that in the sample tube and dividing by the volume of biological fluid taken for analysis, the amount of sodium in 1 cc. of fluid is obtained.

The sodium concentration in 15 normal human blood sera, determined on seven samples, was found to vary between 324 and 343 (average 334) mgm. per 100 cc. Values obtained on 111 photocoloric acid filtrates averaged 3.35 mgm. per 100 cc. Values observed on 3 samples of cerebrospinal fluid were comparable to those observed in blood sera. Samples of low sodium varied in sodium content between 132 and 403 milligrams per 100 cc.

Analysis of known amounts of sodium chloride, both in single aqueous solution and acid with constant amounts of analyzed blood sera, indicated that the error involved in the use of the method varies from -0.5% to $+0.5\%$.

Bibliography

- (1) Alten, F., and Wieland, H.: Colorimetrische Bestimmung des Natriums, Ztschr. f. Pflanzenernähr., Düngung, u. Bodenk. 31A:252-5, 1933.
- (2) Alten, F., Wieland, H., and Hille, E.: Die Bestimmung des Natriums als tri-Uranyl Magnesium Natriumacetat, Ztschr. f. Pflanzenernähr., Düngung, u. Bodenk. 32A: 129-40, 1933.
- (3) Bálint, M. : Eine jodometrische Mikrobestimmung des Natriums, Bioch. Ztschr. 150:424-443, 1924.
- (4) Ball, E.G., and Sadusk, J.F., Jr. : A study of the estimation of sodium in blood serum, Jour. Biol. Chem. 113:661-674, 1936.
- (5) Ball, W.C.: Estimation of sodium and caesium as bismuthinitrites. Part I. Estimation of sodium, Jour. Chem. Soc. 97:1408-1414, 1910.
- (6) Barber, H.H., and Kolthoff, I.M.: A specific reagent for the rapid gravimetric determination of sodium, Jour. Amer. Chem. Soc. 50:1625-1631, 1928.
- (7) Barber, H.H., and Kolthoff, I.M.: Gravimetric determination of sodium by the uranyl zinc acetate reagent. II. Application in the presence of rubidium, caesium, potassium, lithium, phosphate or arsenate, Jour. Amer. Chem. Soc. 51, 3233-3238, 1929.

- (8) Barrenscheen, H.K., and Messiner, L. : Eine kolorimetrische Mikrobestimmung des Natriums, Bioch. Ztschr. 189:308-313, 1927.
- (9) Blanchetière, A.: Sur une méthode de dosage du sodium, Bull. soc. chim. 33:807-818, 1923.
- (10) Brown, H.B. and Shohl, A.T.: The determination of sodium plus potassium as benzidine sulfate, Jour. Biol. Chem. 91:745-749, 1931.
- (11) Bruttini, A.: Determinazione colorimetrica di piccole quantità di uranio nei minerali, Gazz. chim. ital. 23:251-257, 1893.
- (12) Butler, A.M., and Tuthill, E.: An application of the uranyl zinc acetate method for the determination of sodium in biological material, Jour. Biol. Chem. 93:171-180, 1931.
- (13) Caley, E.R.: A new qualitative reagent for sodium, Jour. Amer. Chem. Soc. 51:1965-1969, 1929.
- (14) Caley, E.R.: Determination of true sodium content of calcium carbonate intended for use in J. Lawrence Smith method, Ind. Eng. Chem., Anal. Ed. 1:191-192, 1929.
- (15) Caley, E.R.: The volumetric estimation of sodium, Jour. Amer. Chem. Soc. 52:1349-1353, 1930.

- (8) Barrenscheen, R.K., and Massinger, L.: Eine kolori-
metrische Mikrobestimmung des Natriums, Bloch,
Zschr. 189:308-315, 1937.
- (9) Blanchetière, A.: Sur une méthode de dosage du sodium,
Bull. soc. chim. 23:807-816, 1925.
- (10) Brown, W.D., and Ebel, A.T.: The determination of
sodium plus potassium as peroxide sulfate, Jour.
Biol. Chem. 91:743-749, 1931.
- (11) Brucini, A.: Determinazione colorimetrica di nitrolo
quantità di uranio nei minerali, Gazz. chim. Ital.
23:351-357, 1933.
- (12) Butler, A.W., and Tschiff, E.: An application of the
cupryl zinc acetate method for the determination
of sodium in biological material, Jour. Biol.
Chem. 93:171-180, 1931.
- (13) Carey, E.R.: A new qualitative reagent for sodium,
Jour. Amer. Chem. Soc. 61:1553-1558, 1939.
- (14) Carey, E.R.: Determination of pure sodium carbonate of
calcium carbonate intended for use in J. Distances
with method, Ind. Eng. Chem., Anal. Ed. 1:151-
152, 1929.
- (15) Carey, E.R.: The volumetric estimation of sodium,
Jour. Amer. Chem. Soc. 62:1348-1353, 1940.

- (16) Caley, E.R.: Errors involved in the determination of minute amounts of sodium by the magnesium uranyl acetate method, Jour. Amer. Chem. Soc. 54:432-437, 1932.
- (17) Caley, E.R., and Foulk, C.W.: A gravimetric and colorimetric method for the direct determination of sodium, Jour. Amer. Chem. Soc. 51:1664-1674, 1929.
- (18) Chang, T.-C., and Tseng, C.-L. : Manganese uranyl acetate as a reagent for the detection of sodium, Sci. Quart. Natl. Univ. Peking, 4, 185-189, 1934. (Cited by Woelfel).
- (19) Chen, G.: Microdetermination of sodium by the uranyl zinc acetate method and the titration of uranium with cadmium as the reductant, Jour. Lab. Clin. Med. 21:1198-1202, 1936.
- (20) Das Gupta, P.N.: Use of phenolic acids in the detection, separation, and estimation of metals. Part II. Colorimetric detection and estimation of uranium, Jour. Indian Chem. Soc. 6:763-776, 1929.
- (21) Dobbins, J.T., and Byrd, R.M.: A volumetric method of determining sodium, Jour. Amer. Chem. Soc. 53: 3288-3291, 1931.
- (22) Doisy, E.A., and Bell, R.D.: The determination of sodium in blood, Jour. Biol. Chem. 45:313-323, 1920.

(16) Calley, E.R.: Errors involved in the determination of minute amounts of sodium by the mercuric nitrate-acetate method, Jour. Amer. Chem. Soc. 54:432-437, 1932.

(17) Calley, E.R., and Finkle, C.W.: A gravimetric and colorimetric method for the direct determination of sodium, Jour. Amer. Chem. Soc. 51:1554-1574, 1929.

(18) Chang, T.-C., and Tseung, C.-I.: Manganese in many acetate as a reagent for the detection of sodium, Sci. Quant. West. Univ. Peking, 4, 155-159, 1934. (Cited by Wolfel).

(19) Chen, G.: Microdetermination of sodium by the many acetate method and the detection of uranium with cerium as the reagent, Jour. Lab. Clin. Med. 21:1155-1162, 1935.

(20) Das Gupta, T.N.: Use of phenolic acids in the detection, separation, and estimation of metals, Part II, Colorimetric detection and estimation of uranium, Jour. Indian Chem. Soc. 5:753-756, 1929.

(21) Dobbin, J.F., and Byrd, E.M.: A volumetric method of determining sodium, Jour. Amer. Chem. Soc. 53:2388-2391, 1931.

(22) Doty, W.A., and Hall, E.D.: The determination of sodium in blood, Jour. Biol. Chem. 43:315-325, 1920.

- (23) Duffendack, O.S., Wolfe, R.A., and Smith, R.W.:
Quantitative analysis by spectroscopic methods, Ind.
Eng. Chem., Anal. Ed. 5:226-229, 1936.
- (24) Dulac, J., and Bouat, A.: Dosage volumétrique du
sodium, Ann. école nat. agr. Montpellier 23:191-193,
1935. (Abstract in Chemical Abstracts 30:5144⁹, 1936)
- (25) Elías, A.: Microchemical colorimetric determination
of sodium, Anales asoc. quim. argentina 23:1-3,
1935. (Abstract in Chemical Abstracts 29:7218, 1935)
- (26) Evelyn, K.A.: A stabilized photoelectric colorimeter
with light filters, Jour. Biol. Chem. 115: 63-75,
1936.
- (27) Feldstein, P., and Ward, A.M.: Nickel uranyl acetate
as a qualitative reagent for sodium, Analyst 56:
245-248, 1931.
- (28) Fenton, H.J.H.: Volumetric estimation of sodium, Jour.
Chem. Soc. 73:167-174, 1898.
- (29) Fiske, C.H., and Litarczek, G.: A new method for
potassium, Jour. Biol. Chem. 47: xvi, 1926.
- (30) Furman, N. H., Caley, E.R., and Schoonover, I.C.: The
indirect volumetric determination of sodium based
on the reduction and titration of the uranium in
magnesium sodium uranyl acetate, Jour. Amer. Chem.
Soc. 54:1344-1349, 1932.

- (23) Dufrenoy, O.S., Wolfe, R.A., and Smith, R.W.: Quantitative analysis by spectroscopic methods, Ind. Eng. Chem., Anal. Ed. 5:225-226, 1933.
- (24) Dufrenoy, O.S., and Wolfe, R.A.: Potassium volumetric analysis, Ind. Eng. Chem., Anal. Ed. 5:225-226, 1933.
- (25) Kiser, A.: Microchemical colorimetric determination of sodium, Anal. Assoc. Jour. 1933, 12:191-193, 1933. (Abstract in Chemical Abstracts 30:5147, 1936)
- (26) Kiser, A.: A stabilized photoelectric colorimeter with light filter, Jour. Biol. Chem. 110: 63-70, 1936.
- (27) Kiser, A., and Ward, A.V.: Micro bromine acetate as a qualitative reagent for sodium, Anal. Assoc. Jour. 1936, 12:191-193, 1936.
- (28) Kiser, A.V.: Volumetric estimation of sodium, Jour. Chem. Soc. 1936, 12:191-193, 1936.
- (29) Kiser, A.V., and Lister, R.: A new method for potassium, Jour. Biol. Chem. 110: 63-70, 1936.
- (30) Kiser, A.V., Galey, W.R., and Schindler, I.G.: The indirect volumetric determination of sodium based on the reduction and titration of the bromine in magnesium sodium uranyl acetate, Jour. Amer. Chem. Soc. 58:1544-1547, 1936.

- (31) Gall, H., and Heinig, K.H.: Eine oxydimetrische Bestimmung des Natriums, Ztschr. f. anorg. u. allgem. Chem. 202:154-160, 1931.
- (32) Germuth, F.G., and Mitchell, C.: Detection and identification of specific cations with sodium-alizarin-sulfonate reagent, Amer. Jour. Pharm. 101:46-52, 1929.
- (33) Getman, F.H., and Daniels, F. : Outlines of theoretical chemistry, 5th ed., x + 643 pp., 180 fig. New York: John Wiley & Sons, Inc. 1931.
- (34) Grigant, A., and Boutroux, A. : Le dosage du sodium dans le sérum sanguin, Compt. rend. soc. biol. 104: 872-874, 1930.
- (35) Hald, P.M.: The determination of the bases of serum and whole blood, Jour. Biol. Chem. 103: 471-494, 1933.
- (36) Hoffman, W.S., and Osgood, B.: A photoelectric method for the microdetermination of sodium in serum and urine by uranyl zinc acetate precipitation, Jour. Biol. Chem. 124:347-357, 1938.
- (37) Holmes, B., and Kirk, P.L.: Comments on the microvolumetric sodium method of Ball and Sadusk, Jour. Biol. Chem. 116: 377-380, 1936.
- (38) Jansen, W.H., Heyes, J., and Richter, C.: Die Anwendung der Spektralanalyse zur quantitativen Bestimmung

(31) Gail, H., and Nelson, F.H.: *Die oxymerische*

Bestimmung des Natriums, Elsevier, Amsterdam, 1931.

Allgem. Chem. 208:134-135, 1931.

(32) Gormuth, F.G., and Knebel, G.: *Detection and identifi-*

cation of anionic cations with sodium-sulfate

sulfate reagent, Amer. Jour. Pharm. 101:46-52,

1932.

(33) Gorman, F.H., and Daniels, F.: *Outline of electro-*

chemical chemistry, 2nd ed., p. 843 pp., 1931. New

York: John Wiley & Sons, Inc. 1931.

(34) Grogan, A., and Hargrove, A.: *Le dosage du sodium*

dans le sérum sanguin, Compt. rend. soc. Biol.

104: 872-874, 1930.

(35) Hald, F.M.: *The determination of the bases of serum*

and whole blood, Jour. Biol. Chem. 103: 471-484,

1932.

(36) Hargrove, W.S., and Grogan, A.: *A photometric method*

for the microdetermination of sodium in serum and

urine by means of a potassium precipitation, Jour.

Biol. Chem. 104: 247-257, 1930.

(37) Holmes, E., and Glick, F.L.: *Comments on the electro-*

lysis of sodium method of Hall and Sedgwick, Jour.

Biol. Chem. 104: 257-260, 1930.

(38) Jansen, F.H., Hargrove, W.S., and Richter, G.: *Die Anwendung*

der photometrischen zur qualitativen Bestimmung

- von Alkalien und Erdkalien. III. Mitteilung: Die Mikroanalyse von Natrium im nativen Blutserum, Ztschr. f. physik. Chem. A168:257-273, 1934.
- (39) Jendrassik, L., and Dziobek, L.: Bestimmung des Natriums mittels der Torsionswaage. V. Mitteilung: Biochemische Gravimetriemethoden, Bioch. Ztschr. 287: 262-264, 1936.
- (40) Kahane, E.: Le dosage du sodium par la méthode à l'uranyle, Bull. soc. chim. 47:382-404, 1932.
- (41) Kano, N.: Uses of amalgams in volumetric analysis. IV. Uses of cadmium amalgam, Jour. Chem. Soc. Japan, 43: 332-340, 1922. (Abstract in Chemical Abstracts 16: 2818, 1922)
- (42) Kerr, S.E.: Studies on the inorganic composition of blood. I. The effect of hemorrhage on the inorganic composition of serum and corpuscles, Jour. Biol. Chem. 67:689-720, 1926.
- (43) Kling, A., and Lassieur, A.: Dosage gravimétrique du sodium, Chimie et industrie 12:1012, 1924.
- (44) Kolger, F.: Die Bestimmung von Natrium als Natrium-Magnesium-Uranylacetat, Angew. Chem. 48:561-565, 1935.
- (45) Kolthoff, I.M.: Ein spezifisches Reagens auf Natrium. Ztschr. f. anal. Chem. 70:397-400, 1927.

von Alkalien und Erdsalzen. III. Mitteilung: Die
Mikroanalyse von Natrium im nativen Natrium. Ztschr.
f. physik. Chem. 193:237-273, 1933.

(39) Landrassik, J., and Gubok, L.: Bestimmung des
Natriums mittels der Torsionswaage. V. Mitteilung:

Biochemische Gravimetrie-Methoden. Bloch. Ztschr.

287: 282-284, 1933.

(40) Kahane, A.: La dosage du sodium par la méthode à l'ur-
ange. Bull. soc. chim. 47:363-364, 1932.

(41) Kono, K.: Uses of ammonia in volumetric analysis. IV.
Uses of calcium ammonia. Jour. Chem. Soc. Japan, 43:
232-240, 1932. (Abstract in Chemical Abstracts 18:

2818, 1932)

(42) Kerr, S.S.: Studies on the ionic composition of
blood. I. The effect of hemorrhage on the ionic
composition of serum and corpuscles. Jour. Biol.
Chem. 87:689-720, 1932.

(43) King, A., and Leach, A.: Dosage gravimétrique du
sodium. Chimie et Industrie 12:1012, 1934.

(44) Kolzer, F.: Die Bestimmung von Natrium als Natrium-
Magnesium-Phosphat. Ztschr. f. angew. Chem. 48:581-583,

1933.

(45) Kolthoff, I.M.: Ein spezifischer Reagens auf Natrium.
Ztschr. f. anal. Chem. 70:387-400, 1927.

- (46) Kolthoff, I.M., and Lingane, J.J.: The volumetric determination of uranium with potassium dichromate as reagent and the application of the method to the indirect titration of minute quantities of sodium, Jour. Amer. Chem. Soc. 55:1871-1876, 1933.
- (47) Kramer, B.: Direct quantitative determination of potassium and sodium in small quantities of blood, Jour. Biol. Chem. 41: 263-274, 1920.
- (48) Kramer, B., and Gittleman, I.: An iodometric method for the determination of sodium in small amounts of serum, Jour. Biol. Chem. 62: 353-360, 1924.
- (49) Kramer, B., and Tisdall, F.F.: A simple method for the direct quantitative determination of sodium in small amounts of serum, Jour. Biol. Chem. 46:467-473, 1921.
- (50) Kramer, B., and Tisdall, F.F.: The direct quantitative determination of sodium, potassium, calcium, and magnesium in small amounts of blood, Jour. Biol. Chem. 48:223-232, 1921.
- (51) Lang, R., and Mück, G.: Iodometrische Bestimmung des Natriums als Natriumzinkuranylacetat, Ztschr. f. anal. Chem. 93:100-102, 1933.
- (52) Lattimer, W.M., and Hildebrand, J.H.: Reference book of inorganic chemistry, viii+ 442 pp., illus. New York: The Macmillan Company, 1929.

- (46) Katschhoff, I.W., and Lohmann, J.L.: The volumetric determination of uranium with potassium dichromate as reagent and the application of the method to the indirect titration of minute quantities of sodium. *Jour. Amer. Chem. Soc.* 55:1871-1876, 1933.
- (47) Kramer, B.: Direct quantitative determination of potassium and sodium in small quantities of blood. *Jour. Biol. Chem.* 41: 283-294, 1920.
- (48) Kramer, B., and Gittelman, I.: An iodometric method for the detection of sodium in small amounts of serum. *Jour. Biol. Chem.* 52: 553-559, 1952.
- (49) Kramer, B., and Winkler, F.P.: A simple method for the direct quantitative determination of sodium in small amounts of serum. *Jour. Biol. Chem.* 48:457-472, 1921.
- (50) Kramer, B., and Winkler, F.P.: The direct quantitative determination of sodium, potassium, calcium, and magnesium in small amounts of blood. *Jour. Biol. Chem.* 48:225-232, 1921.
- (51) Lann, R., and Mink, S.: Iodometric determination of sodium as sodium iodide. *Jour. Biol. Chem.* 52:100-102, 1952.
- (52) Lottner, H.M., and Winkler, F.P.: Reference book of inorganic chemistry, VIII 442 pp., Illus. New York: The Macmillan Company, 1939.

- (53) Lewin, A. B. : Volumetrische Bestimmung des Natriums, Ztschr. f. anal. Chem. 104:406-413, 1936.
- (54) Malyarov, K.L., and Yudenich, T.: Colorimetric determination of small quantities of sodium, Zavodskaya Lab. 3:904-6, 1934 (Abstract in Chemical Abstracts 29:2473, 1935).
- (55) Marenzi, A.-D. and Gerschmann, R.: Le microdosage du sodium du sérum ou du plasma, Compt. rend. soc. biol. 114:1212-1213, 1933.
- (56) Marenzi, A.-D. and Gerschmann, R.: La microdeterminación de sodio en plasma o suera. Modificación de la técnica de Salit, Rev. soc. arg. de biol. 9:381-388, 1933.
- (57) McCance, R.A.: Medical problems in mineral metabolism. II. Sodium deficiencies in clinical medicine, Lancet 230: 705-710; 765-770, 1936.
- (58) McCance, R.A.: Medical problems in mineral metabolism. I. Legacies of evolution, Lancet 230: 643-650; 1936.
- (59) McCance, R. A.: Medical problems in mineral metabolism. III. Experimental human salt deficiency, Lancet 230: 823-830, 1936.
- (60) McCance, R. A., and Shipp, H.L.: The colorimetric determination of sodium, Biochem. Jour. 25: 449-456, 1931.

(53) Lewis, A. B. : Volumetric determination of sodium, *Anal. Chem.* 19:400-415, 1938.

Chem. Rev. 19:400-415, 1938.

(54) Matyjaszewski, K. J., and Ydenberg, T. : Colorimetric

determination of small quantities of sodium,

Navodskaya Lab. 3:394-8, 1933 (Abstract in

Chemical Abstracts 28:2475, 1933).

(55) Matyjaszewski, K. J., and Gerschman, R. : The microdetermination

of sodium in serum by the plasma, *Comp. Rend.*

Acad. Sci. Paris 193:1812-1815, 1933.

(56) Matyjaszewski, K. J., and Gerschman, R. : The microdetermination

of sodium in plasma by means of a colorimetric

method, *Rev. Soc. Biol. Paris* 29:1-4, 1933.

ibid. 29:381-388, 1933.

(57) McCance, R. A. : Medical problems in mineral water

drinking, *Brit. Med. J.* 1:1-10, 1935.

Lancet 230:765-770, 1935.

(58) McCance, R. A. : Medical problems in mineral water

drinking, I. The problem of evolution, *Lancet* 230:

822-830, 1935.

(59) McCance, R. A. : Medical problems in mineral water

drinking, II. Experimental human salt deficiency,

Lancet 230:832-835, 1935.

(60) McCance, R. A., and Shipley, H. L. : The colorimetric

determination of sodium, *Biochem. Jour.* 28:

443-456, 1935.

- (61) McCrudden, F.H., and Sargent, C.S.: The determination of sodium and potassium, Jour. Biol. Chem. 33: 235-241, 1918.
- (62) McLean, F.C. and Van Slyke, D.D.: A method for the titration of small amounts of halides, Jour. Amer. Chem. Soc. 37:1128-1134, 1915.
- (63) Miholic, S.S.: The reaction of sodium salts with uranyl acetate alone and in the presence of magnesium, zinc, cadmium, and beryllium, Bull. Acad. Sci. Zagreb. 1920: 16-23, 1920. (Abstract in Chemical Abstracts 15:2595, 1921)
- (64) Mukherjee, J. N. and Ganguly, S.C.: On the effect of dilution on the coagulation of arsenious sulfide hydrosols in its relation to the arsenious oxide content, Jour. Indian Chem. Soc. 7: 465-472, 1930.
- (65) Müller: Eine neue colorimetrische Bestimmungsmethode kleinerer Mengen Uran, Chem. Ztg. 43 : 739-740, 1919.
- (66) Nydahl, F.: Determination of sodium in the presence of the other alkali metals, ammonium and the alkaline earths, Ann. Agr. Coll. Sweden 6:37-87, 1937. (Abstract in Chemical Abstracts 31:8429, 1937)

(61) McGurran, P.H., and Sargent, O.S.: The determination of sodium and potassium, Jour. Biol. Chem. 33:

235-241, 1918.

(62) Nelson, E.G. and Van Slyke, D.D.: A method for the titration of small amounts of halides, Jour.

Amer. Chem. Soc. 37:1128-1134, 1915.

(63) Miholic, S.S.: The reaction of sodium salts with

urea, acetate alone and in the presence of

magnesium, zinc, cadmium, and beryllium, Bull. Acad. Sci. Belges, 1930: 16-23, 1930. (Abstract

in Chemical Abstracts 18:2685, 1931)

(64) Markert, J. K. and Gandy, S.C.: On the effect

of dilution on the coagulation of arsenious

sulfide hydrosols in the relation to the arsenic oxide content, Jour. Indian Chem. Soc. 7:

465-472, 1930.

(65) Müller: Eine neue colorimetrische Bestimmungsmethode

kleinster Mengen Uran, Chem. Ztg. 43: 733-740,

1919.

(66) Wyman, F.: Determination of sodium in the presence

of the other alkali metals, ammonium and the

alkaline earths, Ann. Agr. Coll. Sweden 6:37-39,

1937. (Abstract in Chemical Abstracts 31:5432,

1937)

- (67) Oberst, F. W. : The determination of sodium in human red blood cells, Jour. Biol. Chem. 108:153-160, 1935.
- (68) Ogden, J. B.: Clinical examination of the urine and urinary diagnosis, 2nd ed. 418 pp., 54 fig. Philadelphia, New York, and London: W.B. Saunders & Company, 1903.
- (69) Overman, O. R. and Garrett, O. F.: Determination of sodium. Removal of phosphorous before determining sodium by the uranyl-zinc acetate method. Ind. Eng. Chem., Anal. Ed. 9:72-73, 1937.
- (70) Pearl, R. : Introduction to medical biometry and statistics. 379 pp., 71 fig. Philadelphia and London: W. B. Saunders & Co., 1923.
- (71) Peters, J. P., and Van Slyke, D.D.: Quantitative clinical chemistry. Vol. I. Interpretations, xvi + 1264 pp., 122 fig. Baltimore : The Williams & Wilkins Company, 1931.
- (72) Peters, J. P., and Van Slyke, D.D.: Quantitative clinical chemistry. Vol. II. Methods, xix + 957 pp., 50 fig. Baltimore : The Williams & Wilkins Company, 1931.
- (73) The Pharmacopoeia of the United States of America. Eleventh decennial revision, lxxx + 676 pp. Easton, Pa.: Mack Printing Company, 1935.

(65) O'Brien, P. W. : The determination of sodium in human red blood cells. Jour. Biol. Chem. 108:155-160, 1935.

(66) O'Brien, J. E. : Clinical examination of the urine and urinary diuretics. 2nd ed. 418 pp., 54 figs. Philadelphia, New York, and London: W.B. Saunders & Company, 1935.

(67) Overman, O. R. and O'Brien, J. E. : Determination of sodium. Removal of phosphorus before determining sodium by the uranyl-arsenic acetate method. Ind. Eng. Chem., Anal. Ed. 9:72-75, 1937.

(68) Peters, R. : Introduction to medical chemistry and statistics. 376 pp., VI figs. Philadelphia and London: W. B. Saunders & Co., 1935.

(69) Peters, J. P., and Van Slyke, D.D. : Quantitative clinical chemistry. Vol. I. Intercosmetics, xvi + 1264 pp., 132 figs. Baltimore : The Williams & Wilkins Company, 1931.

(70) Peters, J. P., and Van Slyke, D.D. : Quantitative clinical chemistry. Vol. II. Methods, xix + 654 pp., 50 figs. Baltimore : The Williams & Wilkins Company, 1931.

(71) The Pharmacopoeia of the United States of America. Research department revision, ixix + 876 pp. Easton, Pa. : Mack Printing Company, 1935.

- (74) Poulsson, L.: Über die mikrokolorimetrische Natriumbestimmung, Bioch. Ztschr. 193:423-425, 1928.
- (75) Prinsen-Geerlings, P. F.: Polarographische Natriumbepalungen in Serum, Nederl. Tijdschr. Geneeskunde 81:950-953, 1937.
- (76) Prinsen-Geerlings, P. F. : Polarographische Natriumbestimmung im Blutplasma, Acta Brevia Neerland. Physiol., Pharmacol., Microbiol. 7:38-41, 1937.
- (77) Raszeja, S.: Sur le dosage microvolumétrique du sodium dans le sang, Bull. intern. acad. polon. sci., classe méd. I/II:21-34, 1935.
- (78) Raszeja, S.: Sur le dosage microvolumétrique du sodium dans le sang, Bull. soc. chim. biol. 17:817-830, 1935.
- (79) Rourke, M.D.: On the determination of the sodium content of small amounts of serum or heparinized plasma by the iodometric method, Jour. Biol. Chem. 78:337-344, 1928.
- (80) Russanov, A. K.: Visuelle Spektralmethode zur quantitativen Analyse, von Lösungen, Ztschr. f. anal. Chem. 98:335-342, 1934.
- (81) Rusznyák, S., and Hatz, E.: Eine neue volumetrische Bestimmung von kleinen Mengen Natrium, Ztschr. f. anal. Chem. 90:186-189, 1932.

(74) Porras, L.: Über die mikrokolorimetrische Bestimmung

bestimmung, Bloch. Zschr. 193; 423-425, 1928.

(75) Prinsen-Geerlings, F. F.: Polarographische Bestimmung

bestimmung in Serum, Nederl. Tijdschr. Geneeskunde

81:950-953, 1937.

(76) Prinsen-Geerlings, F. F.: Polarographische Bestimmung

bestimmung im Hämoglobin, Acta Physiol. Neerland.

Physiol., Pharmacol., Microbiol. 7:38-41, 1937.

(77) Rastay, S.: Zur Bestimmung mikrovolymetrisch des Natrium

bestimmung in Serum, Bull. Intern. Acad. Polon. Sci.,

classe 193. 1/11:21-24, 1933.

(78) Rastay, S.: Zur Bestimmung mikrovolymetrisch des Natrium

bestimmung in Serum, Bull. Acad. Polon. Sci., classe 193. 1/11:21-24, 1933.

1933.

(79) Rastay, S.: On the determination of the sodium content

of small amounts of serum or liquor by the method of

the isothermal method, Jour. Biol. Chem. 78:337-342,

1923.

(80) Rastay, S.: Mikrovolymetrische Bestimmung des Natrium

bestimmung von Lösungen, Zschr. f. anal. Chem.

88:333-342, 1934.

(81) Rastay, S., and Rastay, S.: Eine neue volumetrische

Bestimmung von kleinen Mengen Natrium, Zschr. f.

anal. Chem. 90:188-192, 1935.

- (82) Salit, P. W.: A new triple acetate method for sodium determination in biological materials, Jour. Biol. Chem. 96:659-672, 1932.
- (83) Sassier, R.: Sur le dosage volumétrique du sodium en vue de la détermination du rapport Na/Cl dans les urines, Compt. rend. soc. biol. 126:305-307, 1937.
- (84) Sato, M., and Murata, K.: Microdetermination of potassium and sodium in milk, Jour. Agr. Chem. Soc. Japan 13:318-322 (1937). (Abstract in Chemical Abstracts 31:5466, 1937)
- (85) Sheard, C., and Sanford, A.H.: A photoelectric hemoglobinometer. Clinical applications of the principles of photo-electric photometry to the measurement of hemoglobin, Jour. Lab. Clin. Med. 14:558-573, 1933.
- (86) Snell, F.D., and Snell, C.T.: Colorimetric methods of analysis including some turbidimetric and nephelometric methods. Vol. I. Inorganic, 2nd ed. xxiii + 766pp., 109 fig. New York: D. Van Nostrand Company, Inc., 1936.
- (87) Someya, K.: Die Anwendung von flüssigem Amalgam in der volumetrischen Analyse. IV. Über die reduzierende Wirkung von Wismut amalgam, die Reduktion von Uran und die Verwendung von Bichromat-Titration, Ztschr. f. anorg. u. allgem. Chem. 152: 368-381, 1926.

- (88) Stadie, W.C., and Ross, E.C.: A micromethod for the determination of base in blood and serum and other biological materials, Jour. Biol. Chem. 65:735-754, 1925.
- (89) Stoddard, J.L.: An electrolytic method for the determination of sodium plus potassium, Jour. Biol. Chem. 74:677-688, 1927.
- (90) Streng, A.: Über eine neue mikroskopischchemische Reaktion auf Natrium, Ztschr. f. wissenschaftl. Mikroskop. 3:129-130, 1886.
- (91) Thomson, K.B., and Lee, W.C.: The application of spectrographic analysis to the quantitative determination of sodium, potassium, calcium, and magnesium in biological fluids, Jour. Biol. Chem. 118:711-721, 1937.
- (92) Tisdall, F.F., and Kramer, B.: Methods for the direct quantitative determination of sodium, potassium, calcium, and magnesium in urine and stools, Jour. Biol. Chem. 48:1-12, 1921.
- (93) Tissier, M., and Bénard, H.: Sur le microdosage colorimétrique des sels d'urane. Application au dosage du sodium suivant la technique de Blanchetière, Compt. rend. soc. biol. 99:1144-1146, 1928.

(88) Stadler, W.C., and Ross, E.C.: A micro-method for the

determination of base in blood and serum and
other biological materials, Jour. Biol. Chem.
55:733-734, 1933.

(89) Stodard, J.L.: An electrolytic method for the de-

termination of sodium plus potassium, Jour. Biol.
Chem. 74:677-688, 1932.

(90) Ström, A.: Über eine neue mikroskopisch-chemische

Reaktion auf Natrium, Abhandl. d. Wissensch.
Mikroskop. 2:129-130, 1886.

(91) Thomson, K.S., and Lee, W.C.: The application of

spectrophotometric analysis to the quantitative
determination of sodium, potassium, calcium, and
magnesium in biological fluids, Jour. Biol. Chem.
118:771-781, 1937.

(92) Tisdall, F.F., and Kramer, E.: Methods for the direct

quantitative determination of sodium, potassium,
calcium, and magnesium in urine and serum, Jour.
Biol. Chem. 48:1-12, 1931.

(93) Tisser, M., and Ghera, R.: Sur le microdosage

colorimétrique des sels d'urée. Application au
dosage du sodium et du potassium dans le sérum de
lièvre, Compt. rend. soc. Biol. 95:114-116, 1938.

- (94) Treadwell, F.P.: Analytical chemistry. Vol. II.

Quantitative analysis. Authorized translation from the German by W.T.Hall, 4th ed. xi + 926 pp., 126 fig. New York: John Wiley & Sons, Inc., 1915.

- (95) Van Slyke, D.D., Hiller, A., and Berthelsen, K.C.:

A gasometric micro method for determination of iodates and sulfates, and its application to the estimation of total base in blood serum, Jour. Biol. Chem. 74:659-675, 1927.

- (96) Weinbach, A.P.: A micromethod for the determination

of sodium, Jour. Biol. Chem. 110:95-99, 1935.

- (97) Woelfel, W.C.: The colorimetric determination of

sodium as uranyl magnesium sodium acetate, Jour. Biol. Chem. 125:219-227, 1938.

- (98) Yoshimatsu, S.: Colorimetric method for the de-

termination of sodium with 0.1 cc. of serum or blood, Tohoku Jour. Exptl. Med. 8:496-500, 1927.

(94) Treadwell, F.P.: Analytical chemistry, Vol. II.

Quantitative analysis. Authorized translation

from the German by W.T. Burt, 4th ed. xi + 938

pp., 1951. New York: John Wiley & Sons, Inc.

1915.

(95) Van Slyke, D.D., Miller, A., and Bertelson, K.E.:

A gasometric micro method for determination

of iodates and sulfates, and its application to

the estimation of total base in blood serum,

Jour. Biol. Chem. 74:553-575, 1937.

(96) Weinbach, A.P.: A micro method for the determination

of sodium, Jour. Biol. Chem. 110:93-98, 1935.

(97) Woolf, W.G.: The colorimetric determination of

sodium as uranyl manganate sodium molybdate, Jour.

Biol. Chem. 133:219-227, 1938.

(98) Yoshimatsu, S.: Colorimetric method for the de-

termination of sodium with 0.1 cc. of serum or

blood, Tohoku Jour. Exptl. Med. 8:458-460, 1937.



Biographical Note

Matthew Cotton Darnell, Jr. was born at Duckers, Kentucky on June 27, 1913, a son of Matthew Cotton and Ermina (Jett) Darnell. After receiving his elementary education from his mother and in the one-room county school at Duckers, he attended the Frankfort, Kentucky, High School and was graduated in 1929. He received the degree of Bachelor of Science from the University of Kentucky in 1932. From 1932 to 1934 he held a research fellowship in the Department of Agronomy of Massachusetts State College, from which institution he received the degree of Master of Science in 1934. From 1934 to 1936 he was employed as junior chemist by the Memorial Foundation for Neuro-Endocrine Research at Worcester, Massachusetts. From 1936 to 1938 he served as Teaching Fellow in Biochemistry at Boston University School of Medicine, and from 1937 to 1939 as Instructor in Chemistry at the Boston City Hospital School of Nursing.

Biographical Note

Matthew Daniel Dettl, Jr., was born at Danvers, New-
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Science from the University of Kentucky in 1928. From 1928
to 1930 he held a research fellowship in the Department of
Botany of Washington State College, Pullman, Washington.
During his two-year tenure at Washington State he was
the first to find the fungus which he named in honor of the
Daniel Dettl Foundation for Botanical Research at Danvers,
Newbury. From 1930 to 1932 he served as research
fellow in Microbiology at Johns Hopkins University School of Medi-
cine, and from 1932 to 1935 he was instructor in Botany at
the West Virginia State College at Martinsburg.

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